Sepsis in Newborn Infants

Incidence, Antibiotics, Infection Markers

Setyadewi Lusyati

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Stellingen behorende bij het proefschrift

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1. A good surveillance and registration system of neonatal infections saves lives.
   (dit proefschrift)

2. The higher incidence of infections in newborn infants admitted to the NICU of The Harapan Kita Women and Children Hospital, Jakarta, compared to the Beatrix Children's Hospital, Groningen, is due to insufficient hand hygiene and infected IV solutions in Jakarta.
   (dit proefschrift)

3. The emerge of resistant bacteria in a NICU is due to an overuse of antibiotics
   (dit proefschrift)

4. In newborn infants with suspected of late onset sepsis, IL-15 and MIP-1a are good markers to identify a bacterial infection.
   (dit proefschrift)

5. If IL-6 is not increased in the first 24 hours after the start of symptoms compatible with the infection, a bacterial infection is very unlikely and antibiotics can be stopped.
   (dit proefschrift)

6. IL-6 cannot be used to differentiate between sick infants with and without a bacterial infection, as levels are increased in both groups of patients.
   (dit proefschrift)

7. NICU's in developing countries should not be satisfied untill they reach levels of nosocomial infections compatible to levels in developed countries.

8. The care for sick newborn infants always needs balancing. It is like we manage our life.

9. Less treatment is not always easy

10. Stop eating before feeling full (Prophet Mohammad s.a.w)

11. Talent contributes only 15% to achieving success. The rest comes from motivation and determination (Albert Einstein).

12. Sacrifice and consistency will never be wasted (Soejono, my father).

13. There is always a silver lining in the darkness (Pieter J.J. Sauer).

14. Keep focus on one message (Peter H. Dijk)

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Proefschrift

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Dedicated to Tami, Dhia and my parents

Paranimfen : Dr. Peter H. Dijk

Dr. TB. Firmansyah Rifai, Sp.A

Contents

CHAPTER 1

Introduction

The fetus is in utero in an – almost – sterile environment. It is protected against bacteria from the maternal genital tract by membranes in the uterus. Although recent studies show that amniotic fluid is not completely sterile, the bacterial colonization of the fetus mainly takes place during and short after delivery. When membranes are ruptured more than twelve to twenty four hours before the delivery takes place, the vaginal flora may ascent into the uterus and cause a fetal and uterine infection. Despite the rapid and rather massive colonization with bacteria during the passage through the vaginal tract of the mother and from other sources shortly after birth, most newborn infants do not develop an infection. Why some of the infants develop an infection is not completely clear. Maternal antibodies transferred antenatally against bacteria from the mother, most likely play a very important role in the prevention of infections [1]. The infection might occur due to a lack of antibodies as well as a high amount of ingested bacteria. Also, the inhalation of bacteria into the lung might cause a pneumonia that rapidly develops into a sepsis. Not all bacteria present in the maternal genital tract are related to the development of infections [1]. In Table 1 the association between bacteria found in the genital tract of the mother and bacteria causing an infection is shown.

Viral infections can cause clinical symptoms that cannot be distinguished clinically from bacterial infections. Viruses known to cause a sepsis-like disease in newborn infants are Enteroviruses, Herpes viruses and Parvo viruses [1]. The clinical symptoms of an infection by these viruses can be identical to a sepsis caused by bacteria. In this thesis the term sepsis is restricted to infections caused by bacteria and Candida species.

Neonatal sepsis can be divided in two types, depending on the age at onset of symptoms. When the symptoms indicating an infection are detected in the first 72 hours of life, and the infection progresses into a sepsis, it is called Early Onset Sepsis (EOS). These infections are mostly caused by bacteria originating from the mother during the birth process, but colonization shortly after birth also can play a role. Some of the risk factors related to the occurrence of infections are low birth weight, low gestational age, prolonged premature rupture of membranes, fetal anoxia, low Apgar score at 5 minutes and maternal peripartum infection [1,2,3]. When signs of a sepsis start after the third day of life, it is called Late Onset Sepsis (LOS).

These infections are also called hospital acquired or nosocomial infections, as the origin of bacteria causing these infections predominantly originate from the environment where the infant is cared for. The infection can be caused by infected materials used in the care of the infants or bacteria present on the hands of persons touching the infant. Late onset sepsis occurs almost only in infants admitted to a NICU. This can be explained by the often not optimal condition of the infant, the use of invasive procedures and the immaturity of the immune system, present in preterm infants who are most often affected.

The rate of both EOS and LOS is higher in infants admitted to a NICU in developing or underdeveloped countries compared to developed countries. The incidence of EOS in developing countries is 23-50 per 100 infants admitted to a hospital [4,5,6,7] and LOS 17,5-83 per 100 infants as shown in Table 2 [4,6,8,9,10,11,12]. A multi-center study in the USA reported 15,4 infants with EOS and 24 infants with LOS per 100 very low birth weight (VLBW) infants [13]. The rate of both EOS and LOS is strongly related to gestational age and birth weight [14]. A multi-center study in Canada by Aziz et al. showed that the rate of LOS is related to birth weight, and is 23,5 per 100 infants with a weight of less than 1500 g compared to 2,5 in term infants [15]. Cordero et al. found a decrease of EOS episodes (from 12 to 4 per 100 admitted infants) from 1986 to 2002 [16]. In the same period, the incidence of LOS increased from 7 to 31 per 100. The incidence of EOS in developed countries has decreased due to the introduction of antibiotic prophylaxis in mothers who either carry Group B streptococcus or where no test is performed. At the same time, the incidence of LOS due to gram-negative bacteria has increased [17].

The neonatal mortality in developing countries is much higher: 30-42 per 1000 live births compared to 5 per 1000 live births in developed countries. The main causes of mortality are infections, prematurity and asphyxia, infections being one of the most important causes [18]. The mortality rates for newborn infants admitted to a Neonatal Intensive Care Unit (NICU) in developing countries are also much higher than in developed countries. In general, in developing countries, the mortality rate due to an infection during the first three days of life is lower compared to the mortality rates after this period [3]. In contrast, in developed countries, the mortality due to

EOS is three to twelve times higher compared to mortality due to an infection after this period [13].

Table 1. Association between maternal micro flora in the birth canal and infections in the newborn

Bacteria	Association with neonatal disease	
	Significant	Uncommon
S.aureus		+
S. alfa haemoliticus		+
Group A Streptococcus	+	
Group B Streptococcus	+	
Enterococcus	+	
E.coli	+	
Proteus species	+	
Klebsiella species		+
Pseudomonas species		+
Salmonella species		+
Listeria monocytogenes	+	
Anaerobic bacteria		+
Virus	+	
Fungi :Candida albicans	+	

Cited from Klein and Remington et al.,1995 [1]

The pathogens causing an infection are different between developed and developing countries. In developed countries, the main pathogens causing EOS are Group B streptococcus and E. Coli, both most frequently originating from the maternal flora [13,16,17,20]. Coagulase Negative Staphylococci are the most predominant bacteria causing LOS, followed by E.Coli, Klebsiella. and Enterobacter species [13,14,16,21,22]. A review of 11471 cases of sepsis in newborn infants in developing countries showed that Gram-negative bacteria were isolated in about 60% of the infected infants, with Klebsiella sp. as the major pathogen (16-28%) [23]. Almost half of the cases of early onset sepsis were due to Gram-negative bacteria, Klebsiella sp., followed by Pseudomonas and Acinetobacter species [3,4,19,24,25,26]. This might indicate that early onset sepsis in newborn infants in developing countries is more frequently hospital than maternally acquired. Studies from both developed and developing countries show that the mortality rates due to Gram-negative sepsis are very comparable, about 30-50% [28]. Because of the high incidence and the high mortality due to Gram-negative infections, this is a very serious problem in neonatal medicine in developing countries.

A number of studies have investigated why infections occur so frequent in newborn infants. First, the immune system of the newborn infant is not yet fully developed. Both the innate immunity and the ability to respond to infections are limited. The first barriers, i.e., skin and mucous membranes, are easily damaged. When the barrier becomes damaged, an easy path for bacteria is provided. Furthermore, some microorganisms are capable of penetrating these barriers and can thereby gain access to the underlying tissues. There they are encountered by immunological defense mechanisms and may elicit an inflammatory reaction. These defense mechanisms can be nonspecifically directed against a broad range of microorganisms (e.g., neutrophils that phagocytose and kill bacteria) but it may also be specifically directed against a single organism (e.g., antibody-mediated inactivation of the organism). Unfortunately, many elements of the acquired immune system are less developed in the neonate, with gestational and postnatal age among the most important determinants of immune function [1,28,29].

Table 2. The rates of sepsis and main pathogens in NICUs in developing and under developing countries

Year	Country	Author	Study design	Number infected infants /100 infants admitted	GA (wks) or BW (g)	Pathogen
2007	Taiwan	Su BH [9]	Prospective (1 yrs)	LOS=17	35(21-44)	CoNS
2004	Taiwan	Jiang JH [11]	Undefined (9 yrs)	EOS=57 vs 39 LOS=83 vs 17	<37 vs ≥ 37	GBS, E.coli CoNS
2006	South Korea	Sook Jeong [8]	Retrospective (4 yrs)	LOS=30	33,6 ± 3,6	CoNS
2007	Brazil	Couto [10]	Prospective (10 yrs)	LOS=45	All GA	Klebsiella sp. E.coli
2004	Brazil	Silva A [19]	Prospective (2 yrs)	LOS=12 vs 51	<1000 vs >2500	CoNS, Enterobacter sp., S.aureus, K. pneumoniae
2001	India	Karthikeyan [7]	Prospective (6 mo)	EOS=50, LOS=50	All GA	MRSA, Klebsiella sp
2000	Thailand	Petdachai [12]	Undefined (5 yrs)	LOS (VAP)=40	All GA	Acinetobacter, Klebsiella sp Enterobacter sp.
2000	Kenya	Musoke RN [6]	Prospective (4 mo)	All = 17	All GA	Klebsiella, S.epidermidis Enterobacter sp.
1999	Nigeria	Ako-Nai K [5]	Prospective (11 mo)	EOS=47, LOS=52	All GA	S.aureus Pseudomonas
1990	Africa	Nathoo KJ [24]	Prospective (6 mo)	All =21 (per 100 live births)	All GA	GBS, E.coli Klebsiella sp.
1997	Pakistan	Bhuta ZA [26]	Prospective (30 mo)	LOS=45	All GA	Klebsiella sp., Serratia sp. Pseudomonas, S.aureus
1997	Saudi A	Dawodu [3]	Prospective (5 yrs)	7,5 vs 23,8 (per 100 live births)	<1500 vs ≥ 1500	Gram-negatives
1995	Jordania	Daoud AS [25]	Prospective (2 yrs)	LOS=30 vs 24	<1000 vs > 2500	C0NS, Enterobacter sp. Klebsiella. sp.
1995	Malaysia	Lim NL [4]	Prospective (9 mo)	EOS=26, LOS=45,7	All GA	Acinetobacter Klebsiella sp.

Secondly, newborn infants are rapidly colonized by bacteria present in the NICU, often resistant Gram-negative bacteria. Sick newborn infants, especially extremely preterm infants require prolonged intensive care and need intervention with a number of medical devices that might breach physical barriers against infection and thereby facilitate the invasion by nosocomial pathogens. Infants who need mechanical ventilation for more than 8 days have a three-fold higher risk to ventilator associated pneumoniae (VAP) compared to infants without ventilation [9]. Nasal CPAP also increases the risk to develop an infection but at a lower rate than ventilation [27,30]. Other risk factors are central venous catheters (CVC) longer than 7-10 days [9,13,27,30,31,32] and umbilical catheters longer than 5 days [13]. Bladder catheterization is associated with enterobacter septicaemia [33]. Total Parenteral Nutrition (TPN) might cause an infection, either due to contamination of the solution itself, or due to the intravenous lines or CVCs used to deliver the TPN. Use of TPN longer than 20 days significantly increases the risk of infection [9,30,34,35,36,37]. Gastrointestinal pathology and the use of H2 blockers are also shown to be risk factors [9,27], as well as delayed enteral feeds more than 5 days [13]. Finally, contaminated hands are the most important factors in transfering bacteria to the infant. Several controlled trials of hand washing have demonstrated its effectiveness in reducing nosocomial infections [32]. In summary, the smaller the newborn infant, the lower the level of host defense, the higher the rate of medical interventions. All of these together make them vulnerable to develop an infection.

It is very difficult, if not impossible, to diagnose an infection on clinical symptoms. Signs and symptoms of an infection in newborn infants are not specific and may be subtle. These symptoms can be caused by other factors than an infection. Clinical scoring systems are not specific to detect or exclude an infection [2,38,39]. Routine laboratory measurements such as leucocyte count, thrombocyte count, neutrophil count and I/T ratio are not conclusive [40,41,42]. The blood culture remains to be the gold standard to diagnose a neonatal sepsis. The BACTEC system is the method used in most centers, it gives reliable results after 24-48 hours [43]. Results however can be influenced by the procedure to take the blood. The sensitivity and specificity of the blood culture is 100% when blood is taken under recommended procedures. Minimally one mL of blood must be

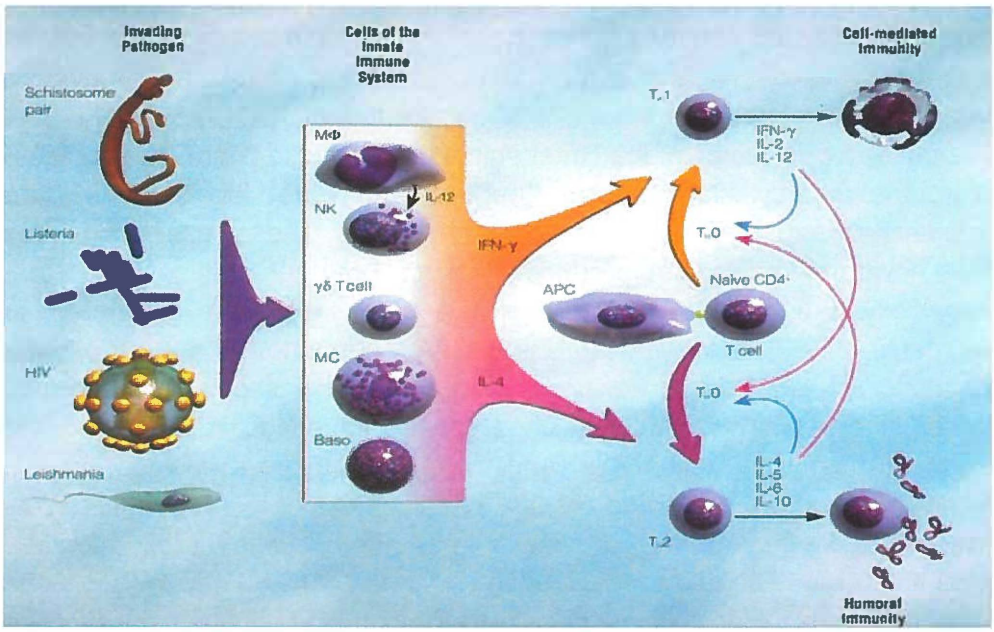
taken under aseptic conditions. Taking blood cultures at multiple sites will not increase the sensitivity [44,45]. In preterm infants, it is often not easy to take one mL of blood. Taking less than one mL of blood or not using aseptic methods can result in false negative or positive results [46]. Another problem especially in developing countries is that the BACTEC system is not always available. C-reactive protein (CRP) is used to detect or exclude a neonatal infection. Some studies have shown that a CRP above 10 mg/dL might be an indication of an infection [40,41,47-50]. Peak values are observed in the first 24 hours after start of the symptoms. The sensitivity of CRP as infection marker is increased when done serially within the first 24 hours after start of the symptoms [50]. However, the CRP level is also increased in infants with signs compatible with an infection but without a positive culture, in patients with meconium aspiration, after birth asphyxia and post surgery. A recent study found that the sensitivity and specificity of CRP to diagnose a sepsis is limited. In 93% of the infants with a proven sepsis was the CRP > 10 mg/dL; however it was also elevated in 73 % of the infants with a suspected sepsis. No increase in CRP was found in 7 % of infants with a proven sepsis and 27 % of the infants with a suspected infection [51]. CRP, therefore, is not a specific indicator of an infection. Procalcitonin (PCT) is another frequently used marker of infection. A recent meta analysis evaluating the use of PCT showed a sensitivity of 70-80 % and a specificity of 80-85 %, depending on the time of measurement after start of the symptoms [52-56].

The generation and maintenance of immunological responses to an infection is controlled by a network of small, nonstructural, intercellular regulatory proteins that mediate a multiplicity of immunologic functions. These so-called cytokines and chemokines (referred to here as cytokines), are induced by specific stimuli, such as several types of bacterial products, and are responsible for the generation, stimulation, and differentiation of multiple cell types as well as for the control and production of other cytokines that may enhance or inhibit the synthesis of protein products and/or biological effects of other cell types and proteins (see Figure 1) [27]. This results in a complex fine-tuned regulatory network that may ultimately succeed in the eradication of the invading microorganism(s). The ability or inability to generate certain cytokines or cytokine patterns in response to infection often determines the clinical course of infection and may greatly

affect the outcome. In certain circumstances, overproduction of cytokines may even lead to shock, multiorgan failure, or death [57,58].

Cytokines might be good markers to differentiate between newborn infants with a sepsis and infants with the same clinical symptoms, without a bacterial infection. Many studies have investigated if cytokines indeed might be able to make this differentiation. Studies on cytokines in newborn infants with neonatal sepsis have been conducted since the late 1990s. Tumor necrosis factor alpha, Interleukin-1, Interleukin-6 and Interleukin-8 were the first cytokines studied that showed higher levels in newborn infants with sepsis compared to controls [59-63]. These results indicated that newborn infants can produce cytokines. Further studies found that especially IL-6, IP-10 and IL-8 might be a potential infection marker in neonates [64-68]. However, there is a wide range in levels and an overlap of these levels between newborns with a bacterial sepsis and controls. Other studies showed that cytokines can be increased in neonates with perinatal asphyxia, mechanical ventilation, or exposure to oxygen [69-71]. Although there are promising results, cytokines are not yet used in clinical practice to differentiate between sick infants with and without and infection. This might partly be due to relative high volumes of blood needed for these measurements. Recently it became possible to analyze twenty five cytokines in 30-50 uL of plasma or serum. This translates to 0,1-0,3 mL of blood, an amount unlikely to contribute to the development of neonatal anemia. This makes it possible to extend studies on the potential role of cytokines in differentiating between infants with and without a sepsis. In Table 3 a list of potential useful cytokines and their producer, inducer and main effect is listed

Figure 1. Simplified scheme of the response of the immune system to an infection



As explained above, a neonatal sepsis is related to severe morbidity and mortality. On clinical symptoms it is impossible to differentiate between sick infants with and without an infection. Laboratory methods are also not specific enough while the results of the gold standard, i.e., the blood culture, are available only after 48-72 hours. Therefore, in clinical practice, antibiotics are given to all newborn infants with symptoms compatible with an infection. In severe sick infants, once antibiotics are given, it is difficult for clinicians to stop them even when the blood culture is negative. Moreover, especially in units with high rates of nosocomial infections, clinicians are afraid not to start antibiotics in newborn infants who receive ventilator support, a chest tube or a central venous line. This all will result in the administration of antibiotics for a rather long period of time in infants that do not need them. The use of antibiotics in a NICU is related to the development of resistance to the antibiotics used [72]. The antenatal administration of ampicillin to prevent Group B Streptococcus (GBS)

infection in the mother and in the newborn infant induced a shift in bacteria causing an EOS, from GBS to Ampicillin-Resistant E.coli [73,74]. Studies from other units reported the same trend [15,75]. Waterer et al. showed that Gram-negative bacteria, members of the Enterobacteriaceae family (E.coli, Enterobacter sp, Klesiella, Citrobacter, Serratia sp.), are often resistant to at least one class of antibiotics that is used as empiric microbial therapy in neonates, including the B-lactams and aminoglycosides [76]. Bizzarro et al. showed that in VLBW infants with LOS, the Methicillin-Resistant Staphylococcus Aureus (MRSA) and Vancomycin-Resistant Enterococci (VRE) are emerging [77]. In developing countries, still, Gram-negative bacteria are the main cause for both early and late onset sepsis. In these countries, 45 to 85 percent of bacteria are resistant to at least one or two of the following antibiotics: ampicillin, ampicillin-sulbactam, aminoglycosides or third generation cephalosporines. The development of resistance to antibiotics might even be a greater threat in developing than in developed countries [7,26,78-81].

The Research Questions investigated in this thesis are:

1. What is the incidence of early (EOS) and late onset (LOS) sepsis, in the Neonatal Intensive Care Unit (NICU) of Harapan Kita Women and Children Hospital, a third level NICU in Jakarta, Indonesia. Which bacteria cause EOS and LOS?

2. Are the solutions used to provide total parenteral nutrition (TPN) one of the causes of these infections in this NICU and can we prevent this by adapting the methods to prepare the TPN solutions?

3. What is the incidence of neonatal infections in a four-year period after the introduction of measures to prevent infections in this NICU?

4. How frequent do an infections occur infants admitted to the NICU of the Beatrix Children's Hospital, Groningen, The Netherlands and how frequent are antibiotics prescribed for a suspected EOS?

5. What are the levels of 25 cytokines during the first week of life in non-infected newborn infants and are they related to gestational age?

6. Can cytokines differentiate between newborn infants with a culture-proven LOS and newborn infants with clinical symptoms of an infection, but a negative culture?

7. Are serial measurements of cytokines during the first 48 hr of life helpful to detect an EOS ?

Table 3. List of cytokines

Cytokines	Main Producer	Inducer	Major effects
IL-1β	Monocytes, Macrophages	Virus, TNFα, pyrogenic exotoxin,	Activates T,B and NK cells (synergistically with IL-2 and IFNα), PMN cells (priming), eosinophils (degranulations)
IL-1Ra	Monocytes, Macrophages, Neutrophils	LPS, IL-4, TNFα, (enhanced by IL-10)	Inhibits: all known effects of IL-1 by competing for binding to the IL-1Ra
IL-2	T-cells	Antigen/mitogen, Pyrogenic exotoxin	Promotes: T cell and B cell growth and differentiation, Ig secretion by B-cells.
IL-4	Naive T-cells, CD4 Th_2 cells	Antigen/ mitogen, IL-2	Promotes : T cells and B cells growth (naive T→ Th_2) , differentiation and proliferation
IL-5	Th_2 cells, mast cells, eosinophils	Antigen/ mitogen	Promotes: B cells and eosinophils differentiation and function
IL-6	Monocytes, Macrophages, Th_2 cells	IL-1, TNFα, IL-4, IFNγ, Viruses, Candida	As IL-1. Stimulates: hepatocytes (acute phase protein production), T cells differentiation and function,
IL-7	Bone marrow, fetal liver cells, intestinal epithalial cell	LPS	Promotes: Pre-T and B cells growth and maturation, NK cell function; Stimulates: tumoricidal activity of monocytes and macrophages ; Secretion of TNFα, IL-1, IL-6 by monocytes
IL-10	T cells, activated monocytes	RSV, LPS. Suppressed by IL-4	Modulates the function of many immunocompetent cells (including T, B, NK cells, monocytes and neutrophils)
IL-12	Monocytes, macrophages, NK cells	Bacteria, parasites, Inhibited by IL-4, IL-10, IL-13	Activates Th_1 induction and maturation, NK cell induction
IL-13	Human activated T cells: CD4,CD3	Activation of Th_2 cells	Promotes: B cells growth and differentiation, IL-1Ra production by monocytes Inhibits : IL-1, IL-6, IL-8, IL-10, IL-12, MIP-1a, GM-CSF, IFNα, TNFα
IL-15	Monocytes, T cells	Cell activation, bacterial infection	As IL-2. Activates : T cells and NK cells
IL-17	Activated (primarily CD^4) T- cells	Mitogen	Act on many cells and tissues in a proinflammatory way. Induce: IL-6 and IL-8 production
TNFα	Monocytes, macrophages, Th_1 cells	Bacterial product, virus,candida	TNFα is a potent endocrine mediator of inflammatory and immune function and protects against infection in general. Induces fever, shock-like syndrome, IL-1 and IL-6

INTERFERON			
IFNα	T and B cells, NK cells, monocytes, macrophages	Viruses, bacteria	Activates :macrophages, NK cells, Anti viral and parasitic activity Inhibits IL-12
FNγ	Virus infected cells, macrophages	Microorganisms	As IFNα
COLONY STIMULATING FACTOR			
GM-CSF	Th_2 cells, eosinophils, neutrophils	Antigen	Activates and diferentiate T lymphocytes,monocytes, neutrophils, eosinophils
CHEMOKINES			
IL-8	Monocytes, macrophages, T cells	LPS, viruses, TNFα, IL-1, Candida albicans	Chemotactic for : neutrophils, T lymphocytes, Activates histamine release
IP-10	T cells, monocytes	Unknown	Chemotactic factor for monocytes and T cells. Promotes T cells adhere to endotelial cells
MIG	Monocytes	Unknown	As IP-10
MIP-1a	T cells, B cells, monocytes, neutrophils, mast cells	Infection,endotoxins	Chemotactic for monocytes (activates IL-1, IL-6, and TNFα production), T cells, neutrophils
MIP-1b	T cells, B cells, monocytes, neutrophils, mast cells	Infection,endotoxins	Chemotactic for monocytes (activates IL-1,IL-6, and TNFα production)
Rantes	T cells, platelet, renal epithelium	Unknown	Chemotactic for monocytes, CD4, eosinophils, basophils
MCP-1	Monocytes, macrophages, B cells	IL-1 , TNFα,	Chemotactic for minocytes (induces macrophages infiltration), stimulates histamine release from basophils
Eotaxine	T cells, platelet, renal epithelium	Unknown	Chemotactic for monocytes, CD4, eosinophils, basophils

Cited from Curfs J et al. [28]

References

1. Klein J and Remington JS. Current concept of infections of the fetus and newborn infant. In: Infectious disease of the fetus and newborn infants. 1995, 4th ed.,1-19 .

2. Geme GW, Murray D, Carter J et al. Perinatal bacterial infection after prolonged rupture of amniotic membranes:an analysis of risk and management. J Pediatrics 1984; 104(4): 608-13.

3. Dawodu A, al Umran K, Twum-Danso K. A case control study of neonatal sepsis: experience from Saudi Arabia. J Trop Pediatr 1997; 43: 84–8.

4. Lim NL, Wong YH, Boo NY, et al. Bacteraemic infections in a neonatal intensive care unit: a nine months survey. Med J Malaysia 1995;50:59–63.

5. Ako-Nai K, Adejuyigbe J, Ajayi V, et al. The bacteriology of neonatal septicaemia in Ile-Ife, Nigeria. J Trop Pediatr 1999;45:146–51.

6. Musoke RN, Revathi G. Emergence of multidrug resistant Gram-negative organisms in a neonatal unit and the therapeutic implications. J Trop Pediatr 2000;46:86–91.

7. Karthikeyan G, Premkumar K. Neonatal sepsis: Staphylococcus aureus as the predominant pathogen. Indian J Pediatr 2001;68:715–17.

8. Sook Jeong I, Sim Jeong J, Ok Choi E. Nosocomial infection in a neonatal intensive care unit South Korea 2006;6:103-110.

9. Su BH, Hsieh HY, Chiu HY et al. Nosocomial infection in a neonatal intensive care unit:a prospective study in Taiwan. Am J Infect Control 2007;35(3):190-5.

10. Couto R, Carvallho E, Pedrosa T et al. A 10-year prospective surveillance of nosocomial infections in neonatal intensive care units. Am J Inf Control 2007;35(3):183-89.

11. Jiang JH, Chiu NJ, Huang FY et al. Neonatal sepsis in the neonatal intensive care unit:characteristics of early vs late onset sepsis. J Microbio Imunol Infect 2004;37:301-06.

12. Petdachai W. Nosocomial pneumonia in a newborn intensive care unit. J Med Assoc Thai, 2000; 83(4): 392-7.

13. Stoll BJ and Hansen N. Infection in VLBW infants:studies from the NICHD Neonatal Research Network. Semin Perinatol 2003;27:293-301.

14. Stoll BJ, Hansen N, Fanaroff A et al. Late onset sepsis in very low birth weight neonates:the experience of the NICHD Neonatal Research Network. Pediatrics,2002;110:285-91.

15. Azis K, McMilan DD, Andrews W et al. Variations in rates of nosocomial infection among Canadian neonatal intensive care units may be practice-related. BMC 2005 (8); 5: 22-9.

16. Cordero L, Rau R, Taylor D et al. Enteric Gram-negative bacilli bloodstream infections:17 years experincesin a neonatal intensive care unit. Am J Inf Control 2004;32(4):189-95.

17. Stoll BJ, Hansen NI, Higgins RD et al. Very low birth weight preterm infantswith early onset neonatal sepsis:the predominance of Gram-negative infections continous in the NICHD Neonatal Research Network. Pediatr Infect Dis J 2005;24(7):635-9.

18. WHO. Neonatal and Perinatal Mortality. Country, Regional and Global Estimates 2000: 1-5.

19. Silva P, Richtmann R, Calil R et al. Healthcare-associated infections among neonates in Brazil. Infect Control Hosp Epidemiol 2004;25:772–777.

20. M Sgro, PS Shah, D Campbell et al. Early-onset neonatal sepsis: rate and organism pattern between 2003 and 2008. J Perinatol 2011: 1–5.

21. Zwet WC, Kaiser AM, van Elburg RM et al. Nosocomial infection in a Dutch Neonatal intensive care unit:surveillance study with definitions for infection specifically adapted for neonates. J Hosp Infect 2005;61(4):300-11.

22. Gordon A, Isaac D. Late onset neonatal Gram-negative bacillary infection in Australia and New Zealand:1992-2002. Pediatr Infect Dis J 2006;25(1):25-9

23. Zaidi A, Huskin C, Thaver D et al. Hospital acquired neonatal infection in developing countries. Lancet 2005;365:1175-88.

24. KJ Nathoo, PR Mason and TH Chimbira. Neonatal septicaemia in Harare Hospital: aetiology and risk factors. Cent Afr J Med 1990;36:150–156.

25. Daoud AS, Abuekteish F, Obeidat A, et al. The changing face of neonatal septicaemia. Ann Trop Paediatr 1995;15:93–6.

26. Bhutta ZA, Yusuf K. Early-onset neonatal sepsis in Pakistan: a case control study of risk factors in a birth cohort. Am J Perinatol 1997; 14: 577–81.

27. Graham P, Begg M, Larson E et al. Risk factors for late onset sepsis in low birth weight infants hospitaliuzed in the Neonatal intensive care unit. The Pediatr Infect Dis J 2006;25(2):113-6.

28. Curfs J, Meis J, Hoogkamp KJ. A Primer on Cytokines: Sources, Receptors, Effects, and Inducers . Clin Microbiol Rev 1997; 10(4):742-80.

29. Tobias S, Andrew C, Peter R et al. Innate immunity in human newborn infants: prematurity means more than immaturity. JMaternal-Fetal and Neonatal Med 2011; 24(1): 25–31.

30. Perlman S, Saiman L, Larson E et al. Risk factors for late onset health care associated bloodstream infections in patients in neonatal intensive care units. Am J Infect Control 2006;35(3):177-82.

31. Chien LY, Macnab Y, Aziz K et al. Variations in central venous catheter related infection risks among Canadian neonatal intensive care units. Pediatr Infect Dis J 2002;21(6):505-11.

32. Clark R, Power R, White R et al. Prevention and treatment of nosocomial sepsis in the NICU. J of Perinatol 2004;24:446-53.

33. Fok TF, Lee CH, Wong E et al. Risk factors for enterobacter septicaemia in a neonatal unit:case control study. Clin Inf Dis 1998;27:1204-9.

34. Tresoldi A, Padoveze M, Trabasso P et al. Enterobacter cloacae sepsis outbreak in a newborn unit caused by contaminated total parenteral nutrition solution. Inf Control Epidemiol 2000;28(3):258-61.

35. Macias A, Munos J, Herera L et al. Nosocomial pediatric bacteriemia:the role of intravenous set contaminaation in developing countries. Inf Control and Hosp Epidemiol 2004;25(3):226-30

36. Macias A, Munos J, Galvan A et al. Nosocomial bacteriemia in neonates related to poor standard of care. Pediatr Infect Dis J 2005;24(8):713-6.

37. Rosenthal V, Maki D, Salomao R et al. Device associated nosocomial infection in 53 intensive care units in 8 developing countries. Annals of internal medicine 2006;145(8):582-91.

38. Okascharoen C, Hui C, Cairnie J et al. External validation of bedside prediction score for diagnosis of late-onset neonatal sepsis. J Perinatol 2007:496-501.

39. Menakshika M, Dutta S, Narang A. Validation of a linical score for the diagnosis of late-onset neonatal sepsis in babies weighing 1000-2500 gram. J Trop Pediatr 2007:54(1):66-9.

40. Russel G, Smyth A, Cooke R et al. Receiver operating characterictics curve for comparison of serial neutrophyl band form and CRP in neonates at risk for infection. Arch Dis Child 1993;67:808-12.

41. Berger C,Uehlinger J, Ghelfi D et al. Comparison of CRP and white blood cell countwith differential in neonates at risk for neonatal septicaemia. Eur J Pediatr 1995;154:138-44.

42. Guida J, Kunig A, Leef K et al. Platelet count and sepsis in very low birth weight neonates:Is there an organism-specific response? Pediatrics 2003;111(6):1411-15.

43. Luke J, Mark WD, Joan F. Incubation time required for neonatal blood cultur become positive. J Paediatr Child Health 2006;42:797–802.

44. Brown DR, Kutler D, Rai B, Chan T, Cohen M. Bacterial concentration and blood volume required for a positive blood culture. J Perinatol 1995;15(2):157-9.

45. Sarkar S, Bhagat I, Christofaro JD et al.A study of the role of multiple site blood cultures in the evaluation of neonatal sepsis. J Perinatol 2005:1-5.

46. Fowlie PW, Schmidt B. Diagnostic tests for bacterial infection from birth to 90 days:a systematic review. Arch Dis Child Fetal Neonatal Ed 1998;78:F92-8.

47. Pourcyrrous M, Bada H, Korones S et al. Significance of serial CRP responses in neonatal infection and other disorders. Pediatrics 1993;92:431-5.

48. Kawamura M, Nishida H. The usefulness of CRP measurement in managing neonatal infection. Acta Pediatr 1995;84:10-10.

49. Franz A, Steinbach G, Kron M et al. Reduction of unnecesary of antibiotic therapy in newborn infants using interleukin-8 and CRP as markers of bacterial infection. Pediatrics 1999;104:447-53.

50. Benitz W, Han M, Madan A et al. Serial serum CRP levels in the diagnosisof neonatal infection. Pediatrics 1998;102(4):E41.

51. Ohlin A, Björkqvist M, Montgomery SM et al. Clinical signs and CRP values associated with blood culture results in neonates evaluated for suspected sepsis. Acta Pædiatrica 2010; 99: 1635–1640.

52. Yu Z, Liu J, Sun Q et al.The accuracy of the procalcitonin test for the diagnosis of neonatal sepsis: A meta-analysis. Scandinavian Journal of Infectious Diseases 2010; 42: 723–733.

53. Janota J,Staranak Z,Belohlavkova S et al. Postnatal increase of procalcitonin in premeture newborns is enhanced by chorioamnionitis and neonatal sepsis. Eur J Clin Invest 2001;31:978-83.

54. Chiesa C, Pellegrini G,Panero A et al. C-Reactive protein,Interleukin-6 and Procalcitonin in the immediate postnatal period:influence of illness severity,risk statuss,antenatal and perinatal complications and infection. Clin Chem 2003;49(1):60-8.

55. Vazzalwar R, Pina E, Puppala B et al.Procalcitonin as a screening test for late onset sepsis in preterm very low birth weight infants. J Perinatol 2005;25:397-402.

56. Lopez J, Solis D, Serradila V et al.Procalcitonin is not insufficiently reliable to be the sole marker of neonatal sepsis of nosocomial origin. BMC Pdiatrics 2006;6(16):1-7.

57. Watkins, L. R., S. F. Maier, and L. E. Goehler. 1995. Immune activation: the role of pro-inflammatory cytokines in inflammation, illness responses and pathological pain states. Pain 63:289–302.

58. Lucey, D. R., M. Clerici, and G. M. Shearer. 1996. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev 9:532–562.

59. Girardin EP, Berner ME, Grau GE et al. Serum tumour necrosis factor in newborns at risk for infections. Eur J Pediatr 1990;149(9):645-7.

60. De Bont, Martens A, van Raan S et al. TNFa,IL-1b and IL-6 Plasma Levels in Neonatal Sepsis.Pediatric Res,1992;380-3

61. Buck C, Bundschu J, Bartmann P et al. Interleukin 6:a Sensitive Parameter for the Early Diagnosis of Neonatal Bacteria Infection.Pediatrics,1994;93:54-8.

62. Ng PC, Chui KM, Fok TF et al. Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecules, and C-reactive protein in preterm very low birthweight infant.Archives of Dis Childhood,1997;77:221-7.

63. Kuster H, Weiss M, Willeitner AE et al. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestations.Lancet,1998; 352(9136):1271-7.

64. Panero A, Pacifico L, Rossi N et al. Interleukin-6 in neonates with early and late onset infection.Pediatr Infect Dis J,1997;16(4):370-5.

65. Maciolek VM, Thijsen SF, Hemels M et al. Inflammatory mediators for the Diagnosis and Treatment of Sepsis in Early Infancy. Pediatric Res 2006;59:457-61.

66. Ng PC, Li K, Wong RP et al. Proinflammatory and anti inflammatory cytokine responses in preterm infants with systemic infections. Arch Dis Child Fetal Neonatal Ed 2003; 88: F209-13.

67. Ng PC, Li K, Chui KM et al. IP-10 is an Early Diagnostic Marker for Identification of Late Onset Bacterial Infection in Preterm Infants. Pediatric Res 2007;61:93-8.

68. Franz AR, Bauer K, Schalk A et al. Measurement of interleukin 8 in combination with C-reactive protein reduced unnecessary antibiotic therapy in newborn infants: a multicenter, randomized, controlled trial. Pediatrics 2004;114(6):1746.

69. Martin C, Boisson C, Haccoun M. Patterns of cytokine evolution (tumor necrosis factor alpha and interleukin -6) after septic shock, haemorrhagic shock and severe trauma. Crit Care Med 1997;25:1813-9.

70. Harris MC, Carl T, Gallagher P et al. Cytokine elaboration in Critically Infants with Bacterial Sepsis, NEC, or Sepsis Syndrome: Correlation with Clinical Parameters of Inflammation and Mortality. J Pediatr 2005;147:462-8.

71. Fotopoulos S, Mouchtouri A, Xanthou G et al. Inflammatory chemokine expression in the peripheral blood of neonates with perinatal asphyxia and perinatal or nosocomial infection. Acta Pediatr 2005;94(6):800-6.

72. de Man P, verhoeven BAN, Verbrugh HA et al. An antibiotic policy to prevent emergence of resistant bacilli. Lancet, 2000;355(18):973-8.

73. Schraag S, Stoll B. Early onset neonatal sepsis in the era of widespread intrapartum chemopriphylaxis. Pediatr Infect Dis J 2006;25(10):939-40.

74. Stoll B, Hansen N, Fanaroff A et al. Changes in pathogen causing early onset sepsis in very low birth weight infants. N Eng J Med 2002;347(4):240-7.

75. Glasgow T, Young P, Wallin J et al. Association of intrapartum antibiotic exposure and late-onset serious bacterial infections in infants. Pediatrics 2005;116:696-702.

76. Waterer GW, Wunderink RG: Increasing threat of Gram-negative bacteria. Crit Care Med 2001;29:N75-N81(suppl).

77. Bizzaro MJ, Gallagher P. Antibiotic resistant organisms in the neonatal intensive care unit. Semin Perinatol 2007;31(1):26-32.

78. Aurangzeb B, Hameed A: Neonatal sepsis in hospital-born babies: bacterial isolates and antibiotic susceptibility patterns. J Coll Physicians Surg Pak 2003;13:629-632.

79. Bell Y, Barton M, Thame M, et al. Neonatal sepsis in Jamaican neonates. Ann Trop Paediatr 2005;25:293-296.

80. Tiskumara R, Fakharee SH, Liu CQ et al. Neonatal infections in Asia. Arch Dis Child Fetal Neonatal Ed 2009 ;94(2):F144-8.

81. Kaistha N, Mehta M, Singla N et al. Neonatal septicemia isolates and resistance patterns in a tertiary care hospital of North India. J Infect Dev Ctries 2010;4(1):55-57.

CHAPTER 2

Neonatal sepsis in a neonatal intensive care unit in Indonesia.

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Abstract.

Background Neonatal mortality is high in developing countries. Neonatal infections contribute not only to mortality, but also cause substantial morbidity. Preventive strategies to reduce the rate of infections and the concomitant use of antibiotics are of key importance. In order to assess efficacy of infection prevention in the NICU in developing countries, assessment of the rate of infections and the use of antibiotics is essential.

Objectives To determine the rate of sepsis on day 1 and day 3-5, the responsible pathogens and the use of antenatal and postnatal antibiotics.

Methods In a tertiary Neonatal Intensive Care Unit (NICU) of Harapan Kita Women and Children Hospital in Jakarta, Indonesia, a retrospective study was conducted in all inborn infants between May 2003 and June 2005. Only inborn infants were evaluated, because outborn and transferred newborn infants might be infected from other sources than the mother. Clinical data of newborn infants, their mothers, including obstetrical risk factors and use of antibiotics were collected. Cultures were taken on admission and between day 3-5 when late onset sepsis was suspected. The incidences of sepsis on day 1 and between day 3-5 were calculated. Statistic analysis used: X^2 test and Fisher exact test.

Results 216 Out of 6600 inborn infants were admitted to the NICU; 133 had symptoms compatible with sepsis and received antibiotics. On day 1, only 9 of 133 cultures were positive. Between day 3-5, 63 out of 74 cultures were positive, despite continued use of antibiotics. A striking occurence of ampicilline-resistant bacteria was found. The majority of organisms were Gram-negative microorganism with Serratia as most prominent. Incidences of sepsis were not related to gestational age. Antibiotics were given to 105 mothers during delivery. No relation between maternal antibiotics and neonatal sepsis was found.

Conclusions. The incidence of sepsis on day 1 in this unit is comparable to centers in developed countries. In contrast the incidence of sepsis on day 3-5 is extremely high. Empiric antibiotics were used in all infants with EOS. Ampicilline-resistant bacteria, i.e. Gram-negative bacteria predominated.

Introduction

In developing countries, neonatal mortality from all causes is about 34 per 1000 live births, most of these deaths occuring in the first week of life[1]. The WHO estimates that infection, prematurity and birth asphyxia are the main causes of death in newborn infants[2,3]. A study from Pakistan showed that the rate of neonatal infection is high in neonatal intensive care unit (NICU) in developing countries, Gram-negative bacteria being most frequent involved[4]. Different studies in Asia and Australia showed that the type ofbacteria causing an infection vary between centers and is influenced by a number of factors[5,6,7,8,9]. One of the most important factors for the development of resistant bacteria is the frequency of antibiotic prescription as well as the types of antibiotics given, either antenatally or postnatally[10]. As part of our efforts to reduce the incidence of neonatal sepsis in our NICU, we studied the incidence of neonatal sepsis on day 1 and day 3-5, the bacteria causing infection and the relation with antenatal and postnatal prescribed antibiotics.

Methods

In this observational study we included all inborn infants admitted to the the NICU of Harapan Kita Hospital, Jakarta, Indonesia, between May 2003 and June 2005. Clinical data of all mothers were recorded. From the infants: birth weight (BW), gestational age (GA), gender and type of delivery were recorded. From the mothers risk factors for infection: rupture of membrane more than 12 hours before delivery, temperature of mothers above 38˚C and abnormalities in the CTG, were recorded. The use of antenatal antibiotics (ampicillin or 3rd generation of cephalosporine) given prior to delivery to the mother either because of caesarian section or when risk factors for an infection were present, was also noted. Neonatal septicaemia was suspected when a newborn infant showed clinical symptoms consistent with an infection (respiratory insufficiency, bradycardia, lethargy, poor feeding, seizure or temperature instability). Blood cultures were analysed with the BACTEC 9240 Rapid Detection System (Becton-Doickson,Sparks, MD) using the PEDS Plus culture bottles and media. When neonatal septicaemia was suspected, 150 mg/kg/day of ampicillin-

sulbactam and 15 mg/kg/day of Amikacin were started immediately. Blood cultures were repeated between day 3-5 in infants with suspected septicaemia without clinical improvement and also when the first culture was negative. Neonatal septicaemia was confirmed by a positive culture. Antibiotics were continued for 14 days in infants with a positive blood culture.

Statistic analysis

The Pearson's chi-square test was used to evaluate the relation between the incidence of sepsis and gestational age. The Fisher exact test was used to evaluate the effect of prescribing antibiotics pre and postnatal on the incidence of sepsis. Statistic analysis was done using SPSS (version 11.0; SPSS, Inc., Chicago, IL).

Results

In the period of May 2003 till June 2005 6600 infants were born in Harapan Kita Hospital, Jakarta. Of these infants, 216 were admitted on the first day of life to the NICU of our hospital. Clinical data of mothers and infants are given in Table 1. In 163 of these 216 infants the mother received antibiotics before delivery. The main reason for giving antibiotics was a caesarean section and /or fever before delivery.

In 133 of the 216 infants an infection was suspected on the first day of life. In all of these infants a blood culture was taken, in nine infants the blood culture was positive. The bacteria cultured are shown in Table 2. Four out of nine positive cultures were due to Serratia species. On day 3-5 an infection was (still) suspected in 74 infants. Of these 74 infants, 63 had a positive blood culture. The cultured bacteria are shown in Table 2: 45 of the 63 positive blood cultures were due to Serratia sp., and 9 due to Klebsiella pneumoniae.

160 Infants of the 216 infants were born by caesarean section. We did not find a relation between infection either on day 1 or day 3-5 with any maternal risk factors, temperature of the mother, abnormal CTG and mode

of delivery (Table 1). There was also no relation to birth weight, gestational age and antenatal antibiotics (Table 3 and 4).

Table 1. Maternal and Neonatal Characteristics of all included infants and mothers, and of infants with Suspected and Proven Neonatal Infection.

Variable	All (n=216)	SNI (n=133)	EOS (n=9)	LOS (n=63)
GA (wks)				
< 30	32	18	0	10
31-34	46	36	2	18
35-<37	37	22	1	10
> 37	101	58	6	25
C-Section	160	104	8	48
Birth weight (g)				
< 1000	6	1	0	1
1000-<1500	30	22	1	9
1500-<2500	84	47	1	23
> 2500	96	63	7	28
Apgar score less than 5 at 5 min	6	2	0	0
Male	141	70	3	28
Mothers :				
< 20 yrs	27	15	1	7
PROM > 12	18	15	0	7
Antenatal antibiotics	163	105	9	49
Temperature >/= 38	15	11	3	6
Abnormality CTG	16	14	3	5

SNI: Suspected Infection on day 1 ; EOS: positive blood culture on day 1 ;
LOS: positive blood culture between day 3-5.

Table 2. Results of all blood cultures taken on day 1 and day 3-5

	Blood culture I (n=133)	Blood culture II (n=74)
Steril	125	11
Total bacteria pathogen:	**9 (EOS)**	**63 (LOS)**
Serratia sp.	4	45
Group B Streptococcus	1	---
Klebsiella pneumoniae	1	9
E. aerogenes	1	7
S. Aureus	2	---
Pseudomonas sp.	---	1
S. pyogenes	---	1

Blood culture I=Blood culture on day 1 ;Blood culture II=blood culture on day 3-5

Table 3: Blood culture on day 1 and day 3-5 in relation to GA

GA (weeks)	BC day 1		p	BC day 3-5		p
	+	-	value	+	-	value
< 30	0	18		10	2	
31 – 34	2	34	**0,509**	18	4	**0,642**
35 - < 37	1	21		10	0	
≥ 37	6	52		25	5	

Using Pearson's chi-square tes. BC: bloodculture; GA, gestational age.

Table 4: Blood culture on day 1 and day 3-5 in relation to antenatal antibiotics

Antenatal antibiotics	day 1 (n=9) BC +	*p* value	day 3-5 (n=63) BC +	*p* value
Yes	7		49	
No	2	**0,204**	14	**0,443**

Using Fisher exact test; BC, bloodculture.

Table 5: Sensitivity patterns of cultured bacteria on day 1 and day 3-5

	Day 1 (n=9)		Day 3-5 (n=63)	
Antibiotics	No.	%	No.	%
Ampicillin	3	37,5	21	33,3
Ampicillin-sulbactam	7	77,8	36	58,1
Gentamycin	5	62,5	55	74,3
Amikacin	5	62,5	52	85,2
Cefotaxime	6	75,0	43	69,4
Ceftazidime	4	50,0	43	69,4
Meropenem	7	87,5	57	91,9

Discussion

We observed in our study an important difference between the rates of sepsis, as indicated by a positive blood culture, between day 1 and day 3-5 in our neonatal intensive care unit. On day 1 we found in 9 out of 216

(4,2%) admitted infants a positive blood culture compared to 63 (29,1%) positive cultures in the same infants on day 3-5.

Infection in newborn infants are often divided in early onset sepsis, diagnosed within the first day of life, and late onset sepsis occuring after the first three days of life.[11] Causes of infection as well as pathogens are different between these two types of infection. Early onset sepsis is mainly caused by perinatally acquired bacteria, Group B Streptococci (GBS), Haemophilus influenzae and E. coli. Group B Streptococci were the most prevalent causes of early onset sepsis in developed countries until perinatal antibiotics were started to prevent these infection. Since then E.coli is the most prevalent cause of EOS.[10-13] Late onset sepsis is considered as nosocomial and caused in most modern neonatal intensive care units by Staphylococci (especially Coagulase-Negative Staphylococci) and Candida species.[11-13]

Stoll et al. recorded - based on the USA Neonatal Research Network - an incidence of early onset sepsis of 15-19/1000 live births of infants with a birth weight of 401-1500g. With the introduction of perinatal antibiotics to prevent Group B Streptococcal disease, the rates of GBS sepsis declined dramatically, but overall incidence of early onset sepsis remained stable. E. coli sepsis increased to 6,8 cases/1000 live births, causing more than half of the neonatal septicaemia.[10] The rates of early onset sepsis in the NICU of the University Medical Center Groningen/Beatrix Children's Hospital is around 0,5 per 1000 deliveries including all gestational ages.[14] In our hospital we observed an incidence of early onset neonatal sepsis of 9 in 6600 deliveries, i.e., 1,4/1000 live births. This slightly higher than the total incidence in The Netherlands. The incidence of early onset sepsis in infants admitted to the neonatal intensive care unit is higher in our hospital compared to the NICUs in developed countries, when taking into account the higher birth weight and gestational age in infants in our NICU. In out unit, 13,5% of the admitted infants had a gestational age less than 30 weeks and less than 1% of the admitted infants had a birth weight < 1000 g. It is well known that the incidence of sepsis increases with a decreasing birth weight and gestational age.

Another difference between results in our NICU and centers in Europe and the USA is the type of pathogen causing the early onset sepsis. [10,13-15] In

our unit Serratia sp., a resistant Gram-negative bacteria, was the main pathogen. It is unclear if infants in our unit were contaminated with Serratia sp. during delivery by the mother or soon after birth in our unit. In our hospital the majority of mothers received a broad spectrum antibiotics before delivery. Whether this has caused an abnormal maternal bacteria flora which is transmitted to the newborn infant is unclear.

We observed a very high incidence of sepsis on day 3-5 in our NICU, almost all caused by Gram-negative bacteria with Serratia sp. as the main organism, followed by Klebsiella pneumoniae. Considering the type of organism causing the infection, these infections must be considered as nosocomial. The postnatal age of our infants developing a nosocomial infection is lower than found in studies in developed countries[16], while the incidence in our unit is much higher. Our results therefore indicate a serious problem with nosocomial infections in our NICU starting soon after admission to the unit. The source of pathogens should be studied to guide infection prevention aimed to reduce nosocomial infections.

The relatively low incidence of positive blood cultures on day 1 might have been influenced by the high rate of antenatal antibiotics given to the mothers. This is in our opinion, however, unlikely. All infants with a positive blood culture at day 3-5 were still receiving antibiotics and nevertheless showed a positive culture. We cannot exclude, however, that a positive blood culture by bacteria other than Serratia sp. might not have been detected due to the use of antibiotics. We suggest studies on alternate methods (not blood culture) to answer this question.

The insensitivity of Serratia sp. found in our infants might be influenced by prescribing antibiotics both antenatal and postnatally. Routine administration of ampicillin to eradicate Group B Streptococcus in developed countries resulted in an emergence of ampicillin-resistant E coli.[10-13] What is happening in developed countries should warn medical practices in developing countries regarding antibiotic treatment. High rates of infection might be ralated to conditions like poor hygiene, low social economic status and malnutrition. In developing countries infections are frequently diagnosed and broad spectrum antibiotics are prescribed for long periods due to fear of infection. Whether these infections are caused by viruses is unknown and perhaps underdiagnosed because viral infections

are diffficult to assess by laboratories in Indonesia. Finally, we could not show an asssociation between antenatal antibiotics and the neonatal infections, but the results might be influenced by the finding that almost all mothers received antibiotics antenatally.

The pattern of sensitivity between the bacteria causing an infection on day 1 and day 3-5 was not different (Table 5). The sensitivity to ampicillin was around 35%, not much different from data from developed countries[15]. The sensitivity of the cultured bacteria to aminoglycosides was around 60-85%, to third generation cephalosporine 50-75%. The most frequent prescribed antibiotics in our unit are ampicillin-sulbactam and aminoglycosides. Cephalosporines are used less frequently.

In conclusion, in this study in a neonatal intensive care unit in Harapan Kita Women and Children's Hospital, Jakarta, we observed an incidence of early onset sepsis only slightly higher than observed in centers in developed countries. The incidence of late onset sepsis on day 3-5, most likely nosocomial infections, was very high. Infection control measures should therefore aim to reduce the incidence of nosocomial infections.

References

1. Costello A, Francis V, Byrne A et al. The states of the world's newborns. Washington: Save the Children Fund, 2001.

2. WHO. Perinatal Mortality. Report No.:WHO/FRH/MSM/967. Geneva: WHO, 1996.

3. Stoll BJ. The Global Impact of Neonatal Infection. Clin Perinatol 1997;24:1-21.

4. Zaidi A, Huskin WC, Thaver D et al. Hospital acquired neonatal infections in developing countries. Lancet 2005;365:1175 – 88.

5. Nathoo KJ, Mason PR, Chimbira TH. Neonatal Septicaemia in Harare hospital: aetiology and risk factors. Centr Afr J Med 1990;36:150-56.

6. Boo NY, Chor CY. Six years trend of Neonatal septicaemia in a large Malaysian maternity hospital. J Paediatr Child Health 1994;30:23-7.

7. Bhutta ZA, Yusuf K. Early Onset Neonatal Sepsis in Pakistan: a case control study of risk factors in a birth cohort. Am J Perinatol 1997;14(9):577-81.

8. Kuruvilla KA, Pillai S, Jesudason M, Jana AK. Bacterial Profile of sepsis in a neonatal unit in a south India. Indian Pediatr 1998;35:851-58.

9. Pessoa-Silva CR, Ricthman R, Calil R et al. Health care associated infection among neonates in Brazil. Infect Control Hosp Epidemiol, 2004; 25: 772-77.

10. Stoll BJ, Hansen N. Infection in VLBW Infants: Studies from the NICHD Neonatal Research Network. Semin Perinatol 2003; 27: 293-301.

11. Tiffany SG, Paul CY, Jordan W. Association of Intrapartum Antibiotic Exposure and Late Onset Serious Bacterial Infection in Infants. Pediatrics 2005;116:696-702.

12. Musoke RN, Revathi G. Emergence of multidrug-resistant Gram-negative organisms in a neonatal unit and the therapeutic implications J Trop Pediatr 2000;46(2):86-91.

13. Staphanie JS, Stoll B. Early Onset Neonatal Sepsis in The Era of Widespread Intrapartum Chemoprophylaxis, Pediatr Infect Dis J 2006;25:939-40.

14. Lusyati S, van de Berg P, Sauer PJJ. Early Onset Sepsis. Pediatric Academic Society Meeting 2006. Abstract 2851.151.

15. Aurangzeb B, Hameed A. Neonatal Sepsis in hospital-born babies: Bacterial isolates and antibiotic susceptibility patterns. J Coll Physicians Surg Pak 2003;13(11):629-32.

16. Stoll BJ, Hansen N, Fanaroff AA, et al. Late onset sepsis in very low birth weight neonatus: the experience of the NICHD Neonatal Research Network. Pediatrics 2002;110:285-91.

17. Sarkar S, Bhagat I, DeCristofaro JD, Wiswell TE, Spitzer AR. A Study of the role of multiple site blood cultures in the evaluation of neonatal sepsis. J Perinatol 2006;26(1):18-22.

CHAPTER 3

A modification in the infusion system that reduced septicaemia in newborn Infants.

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Abstract

Background: Intravenous infusions might be one of the causes of nosocomial infections, particularly in developing countries.

Objective: To evaluate if changing the delivering infusion system reduces the frequency of neonatal sepsis.

Method: Two groups of infants were studied. In group 1 (June-July 2006) the burette system and its injection port was used. This was changed in group 2 (September-October 2006) to a closed system. Outcome parameters were the rate of positive blood cultures during the first 5 days of life.

Result: The rate of sepsis on day 3-5 was significantly higher in group 1 compared to group 2 (37/63 vs 5/53; OR=0.1, CI: 0.03-0.2, $p<0.001$). In group 1, 31 out of 37 positive blood cultures were Serratia sp. compared to 4 out of 5 in group 2.

Conclusions: Changing the i.v. delivery mode significantly reduced the incidence of sepsis. The way i.v fluids are prepared and administered might be an important cause of nosocomial infection in NICUs in developing countries. Infections are one of the main causes of morbidity and mortality in sick newborn infants. The majority of infections are nosocomial. Intravenous infusions might be one of the causes, particularly in developing countries.

Introduction

Infections are important causes of neonatal morbidity and mortality in newborn infants cared for in a neonatal intensive care unit (NICU), especially in developing countries. We found that the incidence of positive blood cultures increased from 9% on day 1 to 63% on day 3-5 in infants admitted to the NICU of Harapan Kita Hospital in Jakarta (1). All these infections on day 3-5 can be considered as nosocomial. Contaminated i.v. solutions might be an important cause of nosocomial infections, especially in NICUs in developing countries (2,3). A number of facts might contribute to this. First, adequate facilities to prepare i.v. fluids and medications might not be available. In optimal situations fluids and medications are prepared in a sterile environment. These conditions are frequently not available in developing countries. In contrast, fluids and medications are prepared within the NICU where the bacterial contamination is widely present. Secondly, due to financial constraints fluids and tubing are not frequently changed and continued to be used after changes in the i.v. fluids are made. A study in the USA indicated that, when properly used, it is unnecessary to routinely replace delivery systems more frequently than every 72 hours (4,5). Due to availability of materials this period is frequently exceeded in our NICU. Finally, not every unit has access to the most modern infusion systems.

We made the clinical observation in our unit that infants given an intravenous solution, either through a central catheter or through a peripheral i.v. line, more frequently developed a nosocomial infection compared to those infants not having an i.v. access even though on the ventilator at same time. In a pilot study we observed that bacteria causing the sepsis were also present in the i.v. solution (Lusyati, unpublished data). The aim of our study is to compare the rate of infections with two different ways of preparing and supplying intravenous solutions. We wondered if making an adaptation to the delivery system, which was possible in our unit and might serve as an example for other units, decreased the incidence of infections.

Subjects and method

The study was conducted in the neonatal intensive care unit of Harapan Kita Hospital, Jakarta, Indonesia. In this tertiary care unit both inborn and out born infants are admitted at an almost equal rate. We compared the rate of infections during the first 5 days of life between two groups of newborn infants in different periods in which a different infusion system was used. For logistic reasons we decided not to use the different infusion systems during the same time period, but to use only one system at one time. The first period was June and July 2006, the second period was September and October 2006, and one month (August 2006) was taken to introduce a new system. The differences between both systems are shown in Table 1. During both study periods all infants admitted to the NICU are included in the analysis. Infants with a fatal congenital anomaly were excluded. In all infants a blood culture was obtained on day 1. A blood culture was repeated on day 3-5 when there were any signs of infection. Blood cultures were analyzed with the BACTEC 9240 Rapid Detection system (Beckton-Dickson, Sparks, MD). Clinical data and intervention devices used were recorded. A combination of Ampicillin-Sulbactam and Amikacin was started on day 1 and continued when infants had clinical deterioration. Further, antibiotics were changed based on the sensitivity pattern of the bacteria from the blood cultures.

Results

During study period 1, 75 infants were admitted to the NICU. Twelve of them were excluded because of either a severe congenital anomaly or the parents refused further treatments. During study period 2, 57 infants were admitted, 4 were excluded. As shown in Table 2, the clinical characteristics of the infants between both groups were not different except the duration of prescribed antibiotics. In group 1, 9 out of 63 infants died of which 2 due to a septicaemia. In group 2, 6 out of 53 patients died of which 3 due to a septicaemia. In group 1, 59 of the 63 patients received antibiotics, compared to 49 out of 53 in group 2. On day 1 no difference in incidence of positive cultures was seen between group 1 and group 2, 11 out of 63 vs. 8 out of 53 (Table 3). In contrast, 37 out of 43 cultures were positive on day

3-5 in group 1, compared to 5 out of 37 cultures in group 2 ($p<0.001$). Thirty-two of the 63 infants in group 1 with a negative culture on day 1 showed a positive culture on day 3-5 compared to 4 out of 45 infants in group 2 (OR=0,017; 95% CI: 0,004-0,73; $p< 0.001$). The mean duration of the use of antibiotics was 13,7 ± 6,8 days in group 1 compared to 9,3 ± 4,9 days in group 2 ($p<0,001$).

Table 1. Two different protocols for preparing and delivering intravenous solutions.

	Group 1 (n=63)	Group 2 (n=53)
Preparation solutions	Within NICU, by nurses, aseptic procedure. Infusion bag and burette system. Additions made through injections into the burette.	Outside of NICU, separated room, by nurses, aseptic procedure. Closed system using 50 mL syringes when infusion rate is less than 5 mL/hr. Burette when infusion rate is more than 5mL/hr.
Adding solutions	On 3rd day electrolytes are added to the infusion by injecting into the burette. When from the start of the infusion. extra glucose or electrolytes are needed, injection in the burette. Amino acids and lipids in separate syringes.	When electrolytes or extra glucose are added, a new solution is made. If a burette is used, nurses are instructed not to do any injection into the burette. Amino acids and lipids in separate syringes.
Changing solutions	Depends on the availability of solutions and systems, usually after 3-7 days. Amino acid and lipids changed every 24-48 hours.	Infusion sets replaced every 3 days, changing not more than once per 24 hours. Amino acids and lipids changed every 24 hours.

The pattern of bacteria causing the infection is given in Table 3. Except for one patient in group 1, all positive cultures were due to Gram-negative bacteria. The most prevalent organism was Serratia sp. with 27 positive cultures on day 3-5 in group 1, compared to 4 cultures with Serratia sp. on day 3-5 in group 2.

We also estimated the expenses of using both infusion systems. The expenses of equipment were slightly higher when using the burette system vs. the closed system (27.6 US $ vs. 35.2 US $). In this calculation we included changing the tubing in the burette system every 7 days, in a closed system every 3 days. Due to the lower incidence of infections, the duration of antibiotics prescribed in group 2 was less compared to group 1. Therefore the costs of antibiotics per patient were less in the group with a closed system compared to a burette system (144.6 US $ vs. 289.3 US $, respectively). Taking this together, the costs of using the closed system when expressed per patient for the first 14 days of life were lower compared to the burette system (179.8 US $ vs. 316.9 US $, respectively).

Table 2. Characteristics of infants between 2 groups

	Group I (n=63)	Group II (n=53)
Male	37	33
Gestational Age (wks)	35 ± 4	36 ± 3
Birth weight (g)	2332 ± 842	2441 ± 897
Died within 5 days of life	9	6
- died due to sepsis	2	3
Infants on ventilation	27	20
Days on ventilation	3 (1-16)	2 (1-22)
Infants on N-CPAP	27	29
Days on N-CPAP	5 (1-29)	4 (1-50)
pH < 7,1 on day 1	4	7
Maintenance infusion (days)	7 (1-39)	6 (1-20)
Amino acid infusion (days)	7 (2-21)	7 (1-18)
Infants with a central venous catheter and sepsis days **of CVC**	11/9 and 5 (2-17)	10/2 and 5 (1-13)
Day starting oral feeding	2	2
Infants with antibiotics	59	49
Duration of antibiotics (days)	13,7 ± 6,8	9,1 ± 4,9

Data are expressed as numbers, median (ranges) or mean ± standard deviation.
N-CPAP: nasal continuous positive airway pressure

Table 3. Bacteria in blood culture on day 1 and day 3-5

	Group I (n=63)		Group II (n=53)	
	day 1	day 3-5	Day 1	day 3-5
Serratia spp.	5	27	4	4
Klebsiella spp.	1	1	0	0
Candida	2	0	1	1
E. aerogenes	1	3	0	0
S. epidermidis	1	1	0	0
E. coli	0	0	1	0
X. malthophilia	1	1	1	0
P. aeruginosa	0	0	1	0
Two bacteria found:				
Serratia sp. & Klebsiella sp.	0	1	0	0
Serratia sp. & E. aerogenes	0	1	0	0
Serratia sp. & X. malthophilia	0	1	0	0
Serratia sp. & Pseudomonas	0	1	0	0
Total positive cultures	11	**37***	8	**5***
Total cultures taken	63	43	53	37

** $p < 0,001$ (fisher exact test)*

Discussion

We observed a striking reduction in infections in infants on day 3-5 of life after changing the procedures of giving i.v. fluids in our NICU in Jakarta, Indonesia. As no other changes in the care of infants were made, this reduction in infections must be due to the change in the i.v. systems. The study lasted only two periods of 2 months. However, we feel both periods are representative for a longer period. In a previous paper we found the same high incidence of infections in our unit during a 2-year period, using the same i.v. systems as in group 1 (1). When we observed the striking decrease in incidence of infections in group 2, this system was implemented in daily care in our unit. From our other data collection, we found that during 2008 only 3 out of 277 admitted patients had a positive blood culture on day 3-5, compared to 33 out of 305 admitted in 2003. Also the overall rate of infections decreased significantly, from 91 out of 305 admitted infants in 2003 to 17 out of 277 in 2008 (Lusyati, unpublished data). This indicates that the low rate of sepsis is persisting and also occurred after the first five days of life.

Gram-negative bacteria are predominant bacteria causing septicaemia in our NICU. Serratia species were the most prominent causes of infections on day 1 as well as day 3-5. Although the overall rate of infection decreased significantly when changing the i.v. solution, Serratia remained the main cause of septicaemia. Gram-negative bacteria including Serratia sp. are present in many NICUs in both developing and developed countries. Many studies investigated the potential source of the infection (6,7,8,9,10,11). One of the potential sources of Gram-negative infections is the intravenous administration of fluids and medication (3). During the 2 months use of a closed system, Serratia presented as the main cause of infection. However, in 2008 no positive bloods cultures, neither on day 3-5 or thereafter, due to Serratia were observed. Most likely Serratia was present at many places in our NICU, and thereby could contaminate the i.v. systems. When we changed the system of preparing and delivering i.v. systems, the reservoir for Serratia infections including the infected patients, decreased. Another contributing factor might have been the reduction in the

duration in the use of antibiotics from a median of 14 days in the group of the burette system, to 9 days in the closed system group.

Umbilical catheters and central venous catheters (CVC) frequently are considered to be sources of infection. There was no difference between both periods in the frequency using these catheters and the time they were used. The CVCs were used for a median period of 5 days. In group 2, two out of 10 patients with a CVC developed a sepsis compared to 9 out of 11 in group 1. It is more likely that this difference is due to contamination of the solutions used than to the use of the CVC itself. The study by Rickard et al. showed that the risk of sepsis is greater when the CVC is used longer than 7 days (12). Unfortunately, in this study we did not culture the infusion solutions. This was due to the relatively high costs of all cultures, culturing the i.v. fluids of all patients over a period of 5 days would have resulted in 580 cultures. No budget was available for this amount.

In this study we changed three important aspects of preparing and delivering the i.v. solutions. First, the preparation was done in the new situation outside the NICU itself, in a separate room. Secondly, a closed system was used, making injections into the system impossible. Finally, i.v. fluids and tubing were changed every three days. It is impossible to conclude which of the measurements have had the most impact. Preparing outside the NICU in a separate room has many advantages (13). Personnel, either nurses but preferably pharmacists, are only busy preparing the intravenous solutions and medications and are not distracted by other tasks. The risk of contamination of the infusion or medication by unwashed hands is more likely to happen if the nurses prepare infusion in the NICU itself (14). Injecting solutions in the injection port of the burette system within the non-sterile environment of the NICU can cause bacterial contamination. This practice is difficult to avoid in a NICU in developing countries with limited resources. Also, due to financial constraints, infusion systems are changed mainly based on availability and costs than on hygienic indication. Different studies evaluated how frequently tubing systems and i.v. fluids should be changed to prevent bacterial contamination. Replacing the burette every 48 hours had a higher of risk of bacterial contamination than once every 24 hours (15). In 1987, Maki et al. published that extending the time to change systems from 1-2 days to 3

days was safe (5). This study, however, found a higher incidence of contaminated systems used for total parenteral nutrition compared to other solutions. This is in accordance with other studies, which found that replacing a tube system every 4-7 days instead of 3 days is safe except for patients receiving total parenteral nutrition (4,16). TPN solutions might be a good culture medium for bacteria. Therefore it is advised to change the systems used for TPN in newborn infants every 24 hours (17,18). The systems used for the other parts of the i.v. might be changed once every 72 ours. Our results indicate that this advice might also be applicable for a NICU in a developing country.

It is important to note that using the closed system the overall costs are not higher than when using the burette system. Due to a marked reduction in the use of antibiotics, overall costs are lower when using the closed system.

In conclusion, we found a striking decrease in nosocomial infections after changing the method of preparing and delivering i.v. solutions. Based on our study we advice other units with a high rate of Gram-negative nosocomial sepsis in developing countries to evaluate the mode of preparing and delivering the i.v. solutions.

References

1. Lusyati S, van den Broek P, Sauer PJ. Neonatal sepsis in a neonatal intensive care unit in Indonesia. J Hosp Infect 2009;71(4):383-5.

2. Macias AE, Munos JM, Galvan A, Gonzales JA, Medina H, Alpuche C et al. Nosocomial Bacteraemia in Neonates Related to Poor Standard of Care. Pediatr Inf Dis J 2005;24:713-6.

3. Macias AE, Munos JM, Bruckner DA, Galvan A, Rodriques A, Guerrero F et al. Parenteral Infusion bacterial contamination in a multi institutional survey in Mexico: consideration for nosocomial mortality. AJIC 1999; 27:285-90.

4. Raad I, Hanna HA, Awad A et al. Optimal frequency of changing the intravenous administration sets: is it safe to prolong use beyond 72 hours? Infect Control Hosp Epidemiol 2001;22:136-9.

5. Maki DG, Botticelli JT, Le Roy ML, Thielke TS. Prospective study of replacing administration sets for intravenous therapy at 48-vs 72 hours interval. 72 hours is safe and cost effective. JAMA 1987;258:1777-81.

6. Reed L, Bulley R, Mc Laughlin B, Bisno AL. Epidemic Serratia marcescens in a NICU: importance of the gastrointestinal tract as a reservoir. Infect Control 1982;3:127-33.

7. Michel YM, Levkoff AH. Endemic Serratia marcescens infection in a neonatal intensive care nursery associated with gastrointestinal colonization. Pediatr Infect Dis J 1985;4:160-7.

8. Villari P, Crispino M, Salvadori A, Scarcella A. Molecular epidemiology of an outbreak of Serratia marcescens in a NICU. Infect Control Hosp Epidemiol 2001;22:630-4.

9. Lai KK, Baker SP, Fontecchio SA. Rapid eradication of a cluster of Serratia marcescens in a NICU: use of epidemiologic chromosome profiling by pulsed gel electrophoresis. Infect Control Hosp Epidemiol 2004;25:730-4.

10. Casolari C, Pecorari M, Fabio G, Cattani S, Venturelli C, Piccinini L et al. A simultaneous outbreak of Serratia marcescens and Klebsiella pneumoniae in a NICU. Journal of Hospital Infection 2005; 61:312-20.

11. Oie S, Kamiya A, Hironaga K, Koshiro A. Microbial contamination of enteral feeding solution and its prevention 2005;12:45-50.

12. Rickard CM, Lipman J, Courtney M, Siversen R, Daley P. Routine changing of intravenous administration sets does not reduce colonization or infection in central venous catheters. Infect Control Hosp Epidemiol 2004;25:650-5.

13. Moore KL, Kainer MA, Badrawi M, Afifi S, Wasfy M, Bashir M et al. Neonatal Sepsis in Egypt associated with bacterial contamination of glucose containing infusion fluid. Pediatr Inf Dis J 2005;24:590-4

14. Moodley P, Coovadia YM, Sturm AW. Intravenous glucose preparation as the source of an outbreak of extended-spectrum beta-lactamase producing Klebsiella pneumoniae infections in the neonatal unit of a regional hospital in Kwa Zulu-Natal. S Afr Med J 2005; 95:861-4.

15. Gorbea HF, Snydman DR, Delaney A, Stockman J, Martin WJ. Intravenous tubing with burettes can be safely changed at 48 hours intervals. JAMA 1984;251:2112-5.

16. Josephson A, Gombert ME, Sierra MF, Karanfil LV, Tansino GF. The relationship between intravenous fluid contamination and the frequency of tubing replacement. Infect Control 1985;6:367-70.

17. Matlow AG, Kitai I, Kirpalani H, Chapman NH, Corey M, Perlman M et al. a Randomized trial of 72- versus 24-hour intravenous tubing set changes in newborns receiving lipid therapy. Infect Control Hosp Epidemiol 1999;20:487-93.

18. Reiter PD, Robles J, Dowell EB. Effect of 24-hour intravenous tubing set change on the sterility of repackaged fat emulsion in neonates. Ann Pharmacother 2004;38:1603-7.

CHAPTER 4

Neonatal sepsis in a NICU in Indonesia : A four-year follow-up study

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Abstract

Background: Recently, we showed that the incidence of late onset sepsis was high in the NICU of Harapan Kita Women and Children's Hospital, Jakarta, Indonesia. We also showed that improvements in the way i.v. fluids were prepared and administered, together with improved attention for hand washing, decreased the incidence of infections.

Objective: To study if the interventions to reduce infections did have a sustained effect.

Patients and methods: Rate of infections, bacteria causing infections and the use of antibiotics were compared between a two-year period (2003-2005) before the interventions, and a four-year period (2007-2010) thereafter. Clinical data of patients were also collected.

Results: The incidence of late onset sepsis was significantly lower in the second compared to the first period, 63 in 216 admitted patients in 2003-2005 vs 63 in 694 infants in 2007-2010. *($p< 0.001$).* The rate of early onset sepsis showed no change. The number of infections due to Serratia sp. decreased significantly, both in late and early onset sepsis. The incidence of early onset sepsis was comparable to data from developed countries, but the incidence of late onset sepsis was also in 2007-2010 higher than in developed countries.

Conclusions: Improving the way intravenous fluids are prepared and administered together with increased attention for hand hygiene has a sustained effect on the incidence of nosocomial infections. These simple and cost-effective interventions might help to reduce infections in other neonatal units in developing countries with a high rate of infections.

Introduction

Infections are important causes of morbidity and mortality, especially in developing countries. In these countries infections in normal term infants are more frequently seen then in developed countries. Infections in NICUs in developing countries also are more frequent compared to NICUs in developed countries. The type of bacteria causing an infection in these units is also different, with a higher percentage of Gram-negative bacteria in developing countries [1-10].

In a previous paper we reported the incidence of infections in 2003-2005 in the NICU of Harapan Kita Women and Children's Hospital in Jakarta, Indonesia [10]. Thereafter we reported that interventions in the method to prepare and administer intravenous solutions decreased the incidence of infections [11]. Previous studies have shown that the rate of infections in the NICU can be reduced by a number of interventions, however, this effect might not always been sustained [12]. Therefore, we assessed the rate of infections, the bacteria causing infections and the use of antibiotics during a 4-year period after changing the way intravenous solutions were made and administered. Results were compared with a 2-year period before these changes were made.

Subjects and methods.

All inborn infants admitted to the NICU of our Hospital between 2007 and 2011 were included in the study. All infants with clinical symptoms compatible with an infection were screened for the presence of an infection. Clinical symptoms included respiratory insufficiency, bradycardia, lethargy, poor feeding, abdominal distention, vomitting, seizures or temperature instability. In all infants routine hematology (leucocytes and differential count, thrombocytes) and CRP were measured at the onset of clinical symptoms. One mL of blood was also taken for a blood culture and analysed with the BACTEC 9240 rapid detection system (BACTON-DICKSON Sparks, ND,USA) using the PEDS + P culture bottles. All infants with a suspected infection received antibiotics, as first choice Ampicillin-Sulbactam (150 mg/kg/day) and Amikacin (15 mg/kg/day). When the

infants did not improve after the start of these antibiotics, a blood culture was repeated and the antibiotics were changed to Meropenem (20 mg/kg/dose). In 2002, the combination of Ampicillin-Sulbactam with Amikacin as first line treatment for sepsis was introduced, therefore the combination Ampicillin with Amikacin was used.

The presence of a positive blood culture was considered a proven sepsis. In this paper we separated between infants with an Early (EOS) and Late Onset Sepsis (LOS), defined as respectively a positive blood culture in the first three days after birth versus the period thereafter. Late onset sepsis most likely is due to colonization with bacteria present in the NICU. This is in contrast to an early onset which is most frequently caused by bacteria from the mother transmitted to the infant during the birth process. However, contamination from sources within the NICU might also cause an EOS, as we found previously that Serratia sp. were the most frequently found bacteria causing an EOS in our unit [10,11].

Interventions

The interventions introduced in our unit in 2006 to reduce the incidence of infections are described before [11]. In short, the methods to prepare and administer i.v. solutions were changed. Secondly, the use of handscrub with 70 % alcohol and chloorhexidine before and after each contact with a patient was strongly re-emphasized and actively monitored.

Statistic analysis

In order to analyse if the intervention had a sustained effect, we used the results of the period 2003-2005, as described before [10] as the reference period. In this study we compare the results of the 4-year period after the intervention with the reference period.

For clinical characteristics we used the Mann-Whitney U test for numeric data and the chi-quadrate test for categorical data. The incidence of sepsis, type of bacteria, rate of antibiotic use between the two periods and rate of antibiotic use in relation to gestational age or birth weight was analysed with the chi-quadrate test.

Results

In the 4-year period between 2007 and 2010, 10681 infants were born in our hospital (Table 1). Of these, 694 infants were admitted to the NICU. There was no trend over time regarding the number of deliveries, the percentage of infants admitted to the NICU, gestational age at birth, birth weight, gender, type of delivery or low APGAR scores (Table 1).

The incidence of suspected and proven EOS was not different between the two time periods. In the period 2003-2005 in 9 out of 216 infants an EOS was found, compared to 10 out of 694 infants in the second period (ns). Almost all infants admitted in both periods received antibiotics directly after birth because of the suspicion of an early onset sepsis. At both time periods, at all gestational ages, around 80% of infants received antibiotics.

There was no change over time in the years 2007-2010 in the incidence of a proven LOS (Table 1). In 2007 21 patients with a proven late onset sepsis were found, compared to 7 in 2008, 13 in 2009 and 22 in 2010. These numbers are significantly lower compared to 63 infants with LOS in 2003-2005 ($p< 0.001$). The decrease in the incidence of LOS was specially found in infants after 32 weeks or more than > 1500 g (Table 2). The incidence of suspected late onset sepsis was not different between both time periods. The incidence of infections in the group infants with mechanical ventilation or without respiratory support was higher in the first vs. the second period. The incidence of infections in infants with long intravenous lines showed the same trend (Table 1).

The organisms causing a sepsis are shown in Table 3. In 2003-2005 four cases of Serratia sp. were found from blood cultures after birth (EOS) compared to 1 Serratia case in 2007 and no Serratia sp. cases thereafter. In 2003-2005 one case of a gram-positive LOS was found, compared to 14 cases in 2007-2010. In contrast, the incidence of Gram-negative infections decreased from 62 out of 216 admitted infants in 2003-2005 to 43 in 694 infants in the second period. In 2007 only 1 case of sepsis due to a Staphylococcus epidermidis was found, with in the same year 9 cases due to Serratia sp. In 2010 the incidence of gram-positive infections had increased causing (41%) of the infections, while only 1 case of Serratia sp., Gram-negative bacteria were responsible for (58%) of the infections.

In Table 4 the resistance to a number of antibiotics for both the gram-positive and Gram-negative bacteria is shown. There was a trend towards an increasing resistance of Gram-negative bacteria against ampicillin/sulbactam and amikacin. In 2003-2005 less than half of the Serratia sp. were resistant to ampicillin/sulbactam. The number of Serratia sp. in the second period is too low to draw conclusions regarding the resistance.

In Table 5 the number of days antibiotics were given in both suspected and proven late onset sepsis is shown. During 2007-2010, infants with a proven sepsis received on average 14-19 days antibiotics, compared to 8-10 days in the group suspected infections.

Table 1. Clinical characteristics of patients

	2003- 2005* (n=216)	2007 (n=184)	2008 (n=158)	2009 (n=190)	2010 (n=162)
Total deliveries	6600	2957	2620	2619	2485
Admitted at NICU	216	184	158	190	162
Gestational age (wks)	36 (26-41)	36 (25-42)	36 (24-42)	36 (25-41)	36 (24-42)
Birth weight (g)	2325 (890-4960)	2280 (510-5235)	2366 (529-5370)	2386(406-4205)	2157 (448-5250)
Male	75	94	98	104	75
AS < 5 at 5 min	22	26	22	44	20
Caesarean section	160	147	122	155	145
Susp. EOS "cases"	133	121	153	180	158
Antibiotics after birth	133	144	149	174	153
Proven EOS"cases"	9	5	4	0	1
Suspected LOS "cases"	88	65	40	55	62
Day of admission when LOS suspected (range)	5 (5-11)	9 (4-26)	10 (4-26)	9 (4-18)	8 (4-28)
Proven LOS "cases"	63	22	7	13	21
Mortality within 7 days	No data	3	3	3	2
N-CPAP (infected)	n.a	98 (4)	93 (1)	98 (3)	96 (4)
Mechanical ventilation (infected)	48 (22)	45 (11)	46 (7)	61 (8)	39 (10)
No resp. support (infected)	88 (17)	41 (2)	19 (0)	31 (0)	27 (2)
IVL (infected)	31 (18)	39 (8)	50 (6)	52 (8)	43 (12)
Infusion system	Semi-open	Closed	Closed	Closed	Closed
Policy "Use alcohol based hand-rub before and after touching nfants"	No	Yes	Yes	Yes	Yes

Data are shown as median (ranges) ; * * Data cyted from Lusyati , 2009 (10)

Table 2 : Incidence of proven late onset sepsis based on BW and GA

	2003-2005**	2007	2008	2009	2010	*p* value*
BW (g)						
≥ 2500	25 (96)	8(81)	3 (74)	2 (88)	3 (64)	**0,000**
> 1500 - < 2500	28 (84)	9 (62)	2 (56)	3 (54)	5 (65)	**0,000**
≤ 1500	10 (36)	7 (41)	2 (28)	8 (48)	13 (33)	0,471
Total	63 (216)	22 (184)	7 (158)	13 (190)	21 (162)	
GA (weeks)						
≥ 37	25 (101)	9 (88)	1 (71)	2 (90)	3 (75)	**0,000**
33-3	28 (81)	2 (54)	2 (55)	4 (57)	9 (49)	**0,002**
≤ 32	10 (34)	11 (42)	4 (32)	7 (43)	10 (38)	0,045
Total	63 (216)	22(184)	7 (158)	13 (190)	21 (162)	

Numbers between brackets are the total admitted infants per group: birth weight (BW) or gestational age (GA)

* p value was calculated by the chi-quadrate test, comparison between period 2003-2005 vs 2007-2010

** Data cited from Lusyati S, 2009 (10)

Table 3: The organisms causing early and late onset sepsis

Organism	2003-2005*	2007	2008	2009	2010	X^2 trend	*p* value**
Early onset sepsis							
Gram-positive	3	2	3	0	1	---	---
Gram-negative	6	3	1	0	0	---	---
Serratia sp.	4	1	0	0	0	--	--
Late onset sepsis							
Gram-positive	1	1	2	3	7	**4,99**	**0,025**
S.Epidermidis	0	1	2	3	5	2,61	0,106
S.Aureus	0	0	0	0	1	--	---
S. Sonei	0	0	0	0	1	--	---
S. pyogenes	1	0	0	0	0	--	---
Gram-negative	62	19	5	9	10	**7,00**	**0,008**
Enterobacter sp.	7	2	2	3	5	1,39	0,238
Pseudomonas sp.	1	2	2	2	0	1,10	0,292
S. Malthopilia	0	2	0	0	1	0,52	0,468
Serratia sp.	45	9	0	3	1	**6,90**	**0,009**
K. pneumoniae	9	2	0	1	1	0,19	0,659
E. Coli	0	1	1	0	1	0,07	0,792
A. Baumanii	0	0	0	0	1	--	---
Candida sp.	0	2	0	1	4	1,08	0,297

*Data cited from Lusyati,2009 (10);

** p value was calculated using the chi-quadrate test

Table 4. Resistance of bacteria to antibiotics used

Pathogen	Years				
	2003-2005*	2007	2008	2009	2010
Gram-negative-bacteria resistant to B Lactam (total Gram-negative bacteria)	26 (62)	11 (19)	0 (0)	7 (9)	8 (10)
Gram-negative bacteria resistant to Amikacine (total Gram-negative bacteria)	9 (62)	6 (19)	0 (0)	2 (9)	6 (10)
Gram-negative bacteria resistant to Meropenem (total Gram-negative bacteria)	5 (62)	3 (19)	0 (0)	2 (9)	1 (10)
Serratia sp. resistant to B-Lactam (total Serratia sp.)	19(45)	1(9)	0 (0)	2 (3)	1 (1)
Serratia sp. resistant to Meropenem (total Serratia sp.)	4 (45)	0 (9)	0 (0)	0 (3)	0 (1)
S.epidermidis resistant to B-Lactam (total gram positive)	0 (0)	1 (1)	1 (2)	1 (3)	4 (5)

*Data cited from Lusyati,2009 (10)

Table 5. Duration (days) of antibiotics prescribed in proven and clinical late onset sepsis

	Years			
	2007	2008	2009	2010
Proven-LOS				
First line : Ampicillin – Sulbactam	9,6	5,8	3,2	5,4
Amikacin	6,1	4,8	3,2	6,5
Second line : Meropenem	9,6	7	12,6	10
Duration antibiotic per patient	19,2	12,8	15,8	15,4
Clinical-LOS				
First line : Ampicillin – Sulbactam	5,4	5,2	2,9	4,4
Amikacin	4	5,4	2,9	4
Second line : Meropenem	3,5	4,8	5	3,3
Antibiotic duration per patient	8,9	10	7,9	7,7

Discussion

In a previous study we described that in 2003-2005 in 63 out of 216 infants admitted to our NICU developed a late onset sepsis. After making changes in a way the TPN solutions were prepared, their mode of administration and focused attention for proper hand hygiene, the incidence of LOS decreased to 63 out of 694 infants. The incidence of LOS in a 4-year period after these interventions remained at the lower level observed directly after the intervention (11). The incidence in EOS showed no change, but infections due to Serratia sp. disappeared.

The incidence of Early Onset Sepsis in the years 2007-2010 in our unit was 1.4 per 100 admitted infants or 0.9 per 1000 live birth. This incidence is comparable to data found in developed countries (13,14,15).

Despite the reduction in the incidence of LOS in our unit, the incidence is still higher compared to developed countries. A recent study in Canada (14) showed an incidence of LOS of 23,5 per 100 admitted infants with a mean gestational age of 29 weeks and a birth weight of 975 grams, compared to 31 infants per 100 infants with a gestational age of 30-32 weeks in our unit in 2003-2005 and 32 out of 155 in 2007-2010 (25 per 100 infants).The incidence of LOS showed a sharp decrease with increasing gestational age in the study from Canada, the incidence in infants with a birth weight of more than 1500 g was 2.3 per 100 infants. In our unit the incidence in infants with a birth weight of more than 1500 g, was 28.8 per 100 infants in 2003-2005 and 5,4 in 2007-2010. Our efforts to improve the preparation and administration of infusions together with aggresively promoting hand hygiene reduced the incidence of neonatal sepsis in newborn infants, especially in infants of more than 32 weeks and a birth weight of more than 1500 g. However, the incidence of sepsis remained slightly higher in all groups compared to the incidence in developed countries. One reason for the higher incidence might be that the awareness of at least of the personal entering the NICU to perform proper hand hygiene is not optimal. Also, there is a lack of sterilization equipment for systems like the filter of the ventilator.

The bacteria causing a LOS seem to shift during the last four years. In the years 2003-2005 Gram-negative bacteria and predominantly Serratia sp. were the main causes of infection (10). In 2007 Gram-negative infections and in particular Serratia sp. were still prevalent . In contrast, in 2009-2010 there were no infections caused by Serratia sp, while there was an increase in Staphylococcus epidermidis infections. These bacteria are the most frequent cause of LOS in NICUs in developed countries. This is related to the use of long intravenous lines and the use of TPN (16,17). We did not, however, observe a change in the use of TPN or long i.v. lines in the past four years in our unit. Why in our unit the incidence of Staphylococcus epidermidis increased needs further study. The reduced incidence of Serratia sepsis might be related to improvements in the preparation and

administration of TPN solutions. The improved hand hygiene will certainly also have contributed to this decrease. High infections with Serratia sp. is found to occur in clusters. The level of colonisation of a unit with these bacteria might fluctuate. A temporary decrease in Serratia sp. in our unit is, in our opinion less likely due to the high and persistent incidence of infections in 2003-2005, and a persistent low incidence in 2008-2010.

The use of antibiotics in our unit still gives reason for concern. Almost all infants received antibiotics after birth. The threshold to start with antibiotics is low. The duration of antibiotics given for a proven sepsis is in accordance with most recommendations (18). The duration of antibiotics given for suspected sepsis was rather long. As this study was observational, we did not intervene in the clinical use of antibiotics.

The frequent use of antibiotics might explain the resistance to the antibiotics used as first line treatment in our unit. The combination of ampicillin/sulbactam with amikacin was introduced in 2002, before that time ampicillin was given together with amikacin. In 2003-2005, more than 50% of the Gram-negative bacteria were sensitive to ampicillin/sulbactam, but this dropped to 20% in 2009-2010. The sensitivity to amikacin also seems to decrease. Other studies have shown that the resistance of bacteria is related to the type of antibiotics used in a NICU (19-24).

In conclusion, the rate of Early Onset sepsis in our unit in Indonesia was 3.3/100 admitted infants, comparable to data found in units in developed countries and did not show a marked change over the past eight years. The incidence of Late Onset Sepsis decreased significantly after changing the way i.v. fluids were prepared and administered. This change was maintained over a four year period. In that period, the incidence of infections due to Gram-negative bacteria decreased while the incidence of gram-positive bacteria, especially staphylococcus epiderdimis, increased. We do not have an explanation for this increase in gram-positive infections, as there were no changes in the population of newborn infants or in the use of long i.v. lines. Finally, the use of antibiotics in our unit remains at a high, and probably dangerous level. Measurements that can help clinicians in the decision either not to start antibiotics or to stop within days after initiation in case there is no bacterial infection are urgently needed.

References

1. Dawodu A, al Umran K, Twum-Danso K. A case control study of neonatal sepsis: experience from Saudi Arabia. J Trop Pediatr, 1997; 43: 84–8.

2. Zaidi A, Huskin C, Thaver D et al. Hospital acquired neonatal infection in developing countries. Lancet,2005;365:1175-88.

3. Lim NL, Wong YH, Boo NY, et al. Bacteraemic infections in a neonatal intensive care unit: a nine months survey. Med J Malaysia 1995;50:59–63.

4. Ako-Nai AK, Adejuyigbe EA, Ajayi FM, et al. The bacteriology of neonatal septicaemia in Ile-Ife, Nigeria. J Trop Pediatr 1999;45:146–51.

5. Musoke RN, Revathi G. Emergence of multidrug resistant Gram-negative organisms in a neonatal unit and the therapeutic implications. J Trop Pediatr 2000;46:86–91.

6. Karthikeyan G, Premkumar K. Neonatal sepsis: Staphylococcus aureus as the predominant pathogen. Indian J Pediatr 2001;68:715–17.

7. Jiang JH, Chiu NJ, Huang FY et al. Neonatal sepsis in the neonatal intensive care unit:characteristics of early vs late onset sepsis. J Microbio Imunol Infect,2004;37:301-06.

8. Petdachai W. Nosocomial pneumonia in a newborn intensive care unit. J Med Assoc Thai, 2000; 83(4): 392-7.

9. Silva P, Richtmann R, Calil R et al. Healthcare-associated infections among neonates in Brazil, Infect Control Hosp Epidemiol 2005;25: 772–777.

10. Lusyati S and Sauer PJ. Neonatal sepsis in a neonatal intensive care unit in Indonesia. J Hosp Infect 2009;71(4):383-5.

11. Lusyati S, c.v Hulzebos, Sauer PJ. A modification in the infusion system that reduced septicaemia in newborn infants. J Trop Pediatr. 2010 Apr;56(2):132-3.

12. Cordero L, Rau R, Taylor D et al.Enteric Gram-negative bacilli bloodstream infections:17 years experincesin a neonatal intensive care unit. Am J Inf Control,2004;32(4):189-95.

13. Stoll BJ, Hansen N, Fanaroff A et al. Late onset sepsis in very low birth weight neonates:the experience of the NICHD neonatal Research Network. Pediatrics,2002;110:285-9.1

14. Azis K, McMilan DD, Andrews W et al. Variations in rates of nosocomial infection among Canadian neonatal intensive care units may be practice-related. BMC, 2005 (8); 5: 22-9.

15. Stoll B and Hansen N. Infection in VLBW infants:studies from the NICHD Neonatal Research Network. Semin Perinatol 2003;27:293-301.

16. Perlman S, Saiman L, Larson E et al. Risk factors for late onset health care associated bloodstream infections in patients in neonatal intensive care units. Am J Infect Control 2006;35(3):177-82.

17. Chien LY, Macnab Y, Aziz K et al. Variations in central venous catheter related infection risks among Canadian neonatal intensive care units. Pediatr Infect Dis J 2002;21(6):505-11.

18. Cloherty JP, Eichenwald EC, Stark AR. Bacterial and fungal infection. In: Manual of Neonatal Care. 4th ed.,2004: 287-312.

19. P de Man, B A N Verhoeven, H A Verbrugh et al. An antibiotic policy to prevent emergence of resistant bacilli. Lancet, 2000; 355(18): 973-8.

20. Schraag S, Stoll B. Early onset neonatal sepsis in the era of widespread intrapartum chemopriphylaxis. Pediatr Infect Dis J 2006;25(10):939-40.

21. Stoll B, Hansen N, Fanaroff A et al. Changes in pathogen causing early onset sepsis in very low birth weight infants. N Eng J Med 2002;347(4):240-7.

22. Glasgow T, Young P, Wallin J et al. Association of intrapartum antibiotic exposure and late-onset serious bacterial infections in infants. Pediatrics 2005;116:696-702.

23. Bizzaro MJ, Gallagher PG. Antibiotic resistant organism in the neonatal intensive care unit. Semin Perinatol 2007;31(1):26-32.

24. Waterer GW, Wunderink RG: Increasing threat of Gram-negative bacteria. Crit Care Med 2001;29:N75-N81 (suppl).

CHAPTER 5

Septicaemia and the use of antibiotics in the first days of life

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Abstract

Background: Antibiotics are frequently prescribed to newborns for fear of early onset sepsis (EOS), occurring in the first 2 days of life. However, exact data on this presumed overuse of antibiotics are largely unknown., whereas the benefits of this practice have to be balanced against side effects like fungal infections and antibiotic resistance.

Objectives: To analyze the incidence of (suspected) EOS and use of antibiotics in newborn infants.

Methods: A restropective study of all inborn infants admitted to the NICU of the Beatrix Children's Hospital, University Medical Center Groningen, The Netherlands, was conducted between January 2003 and December 2004. Rates of blood culture-proven EOS and antibiotic treatment for suspected EOS were collected. Maternal risk factors (PROM, fever, use of corticosteroids and antibiotics) and infant variables (birth weight, gestational age, Apgar Score, C-reactive protein, leucocytes, immature: total neutrophil ratio (I/T-ratio), glucose and blood gas analysis) were retrieved from a local database.

Result. 662 inborns were admitted to the NICU. In 467 infants EOS was suspected. 423 infants were treated for suspected EOS. Ten (1,5%) infants had blood culture-proven EOS, 11 microorganisms were isolated: three Gram-negative organisms and eight gram-positive organisms The incidence of EOS was comparable at all gestational ages. Antibiotic treatment was prescribed more often to infants with a gestational age less than 32 weeks. The number of infants treated for one case of EOS was 68 in the group infants with a gestational age between 24-27 weeks compared to 21 in infants with a gestational age between 37-42 weeks.

Conclusion. EOS is frequently suspected and newborn infants are often treated with antibiotics. However, EOS is uncommon with an incidence of 1,5/ 100 admitted infants. For each infants with EOS 42 infants are treated with antibiotics. The "overuse" of antibiotics is not only restricted to infants born with a very low gestational age.

Introduction

Bacterial sepsis in the newborn is a severe and life-threatening disease. The mortality when untreated is around 100% and varies between 5% and 50% after treatment (1-5), with the highest rates in preterm infants. The success rate of antibiotic treatment depends on the early initiation of appropriate antibiotics and the invasiveness of the bacteria causing the infection. The first clinical symptoms of a sepsis are non-specific and may be subtle. The presence of maternal risk factors like maternal fever, chorioamnionitis and prolonged rupture of membranes may be related to, but do not predict the predict the presence of an early onset sepsis (EOS), defined as a sepsis starting within the first 2 days of life. Absence of these risk factors does not rule out an EOS.

The golden standard to detect a neonatal sepsis is a blood culture. A positive blood culture is a definite proof of infection, a negative blood culture has a high negative predictive value (6-8). The results of a blood culture is definite only after 48-72 hours. Given the difficulty of the clinical diagnosis of EOS, the potential fatal consequences of delayed treatment (for morbidity and mortality) and delayed results of the blood culture, it is recommended to start antibiotic therapy in all infants with suspected EOS. This approach will probably cause, in retrospect, "unnecessary" treatment in many infants.

The wide-spread use of antibiotics has drawbacks. It will lead to the emergence of resistant bacteria in the NICU (9). In the newborn itself it may lead to an abnormal colonisation of the gastro-intestinal tract, to development of resistant bacteria in the patient itself, and potentially to a higher incidence of yeast infections during the newborn period (9-11). A restrictive use of antibiotics in a NICU is therefore needed. It is unclear at this stage if the "over-use" of antibiotics is restricted to infants born with a very low gestational age. It is important to know in which group of patients overuse is most prevalent and where reductions would have the most benefit. This information is also important for designing studies aimed at detecting predictive factors for EOS in order to reduce any overuse of antibiotics.

The aim of this study is to analyze the incidence of suspected EOS, the use antibiotics for EOS and the proven incidence of EOS in a cohort of all newborn infants admitted to one NICU in a developed country. Secondly, all parameters are related to the gestational age of the infants.

Methods

A restropective study of all inborn infants admitted in the first 2 days of life to the NICU of Beatrix Children's Hospital, University Medical Center Groningen, The Netherlands between January 2003 and December 2004 was conducted.

This tertiary NICU covers an area with 1.7 million inhabitants and 20.000 births per year. In the Netherlands, obstetricians and pediatricians agreed on a protocol for antenatal and postnatal indications for transport (12). When it is expected that the newborn infant will have to be admitted to the NICU postnatally, the pregnant mother is referred antenatally to the NICU of a perinatal center. According to this protocol all mothers expected to deliver before 32 weeks are transferred. In The Netherlands there are in total 10 perinatal centers with a NICU for a population of 16 million inhabitants. No children were excluded from the study. Clinical information about the newborn infants was obtained from a central database, information on blood cultures from the microbiological database and maternal characteristics were obtained from an obstetrical database.

Early onset sepsis was defined as a positive blood culture taken within 48 hours after birth. Cultures positive for organisms considered as contaminants (such as *corynebacterium*) were excluded. In case of a positive culture with coagulase-negative staphylococci the chart was reviewed to distinguish between a definite infection or contamination. Definite infection with coagulase-negative staphylococci was defined as two positive blood cultures within 48 hours with the same pathogen or by one positive blood culture and a C-reactive protein level > 10 mg/L within two days after taking the blood culture. The diagnosis suspected early onset sepsis was made when a blood culture was taken or antibiotics were

prescribed within the first 48 hours after birth. All decisions were made by the attending physician.

Blood was drawn at least 0.5-1 mL of blood from a peripheral vein, after cleaning the skin with 0.5% chloorhexidine in 70% alcohol for blood culture. Blood cultures were analyzed with the BACTEC 9240 Rapid Detection System (Becton-Dickson, Sparks, MD) using the PEDS Plus culture bottles and media. Hemoglobin, C-reactive protein, leucocyte count, thrombocytes, segmented neutrophilis, non-segmented neutrophils, glucose, pH and standard base excess were retrieved from the laboratory database. The IT ratio was calculated by dividing non-segmented neutrophils, i.e., immature neutrophils: total neutrophils ratio. The pH from the umbilical artery and vein was taken from the obstetrical database. Results of the laboratory tests were considered to be normal when results were within the reference range of our hospital. When two or more tests were done and one test was outside the reference range, the result of the test outside the reference range was used for the analysis.

Statistic analysis

Antenatal risk factors (PROM, fever, corticosteroids, route of delivery, urinary tract infection, meconium stained amniotic fluid and antibiotic use) and infant variables (birth weight, gestational age, Apgar score, intrauterine growth, mortality in first week and laboratory tests) were analyzed as binary variables with chi square or Fisher's exact test as appropriate. *P* values of < 0.05 were considered significant. Statistical analyses were performed with SPSS version 13.0 software (SPSS, Chicago, IL).

Results

Clinical data of the mothers and infants included are given in Table 1. Out of the 662 admitted infants 467 infants were suspected to have early onset sepsis. In 427 infants a blood culture was taken in the first two days of life, in 423 infants antibiotics were given. In infants with a gestational age < 28 weeks 96% of the admitted infants were suspected to have early onset sepsis, in term infants 54%. No association was found between EOS and the maternal risk factors, antenatal steroids, antibiotics, mode of delivery,

maternal fever, urinary tract infection or meconium stained amniotic fluid (Table 2). No association was found between EOS and birth weight, gestational age, gender and Apgar score at 5 minutes (Table 2).

In 21 of the infants the blood culture taken in the first 48 hours of life was positive. Eleven blood cultures were considered contaminant according to the definition, so ten of the 662 infants were diagnosed to have EOS. From these ten infants, 11 pathogens were isolated: Two Gram-negative and nine gram-positive micro-organisms (Table 3). Nine out of the ten infants with EOS survived in the first week of life, one infant died to a fulminant E.Coli sepsis on the first day of life. When the infants were devided in groups according to gestational age, we did not observe a difference in the incidence of EOS between these groups. The rate of suspected EOS increased with a longer gestational age. Therefore the ratio of EOS per suspected EOS was higher with increasing gestational age.

Out of the 662 admitted infants 423 (64%) infants were treated with antibiotics for suspected EOS (Table 4). Antibiotic use was higher among infants with a shorter gestational age: 93% of the infants with a gestational age < 28 weeks received antibiotics compared to 34% of term infants. A total number of 423 infants received antibiotics, and 413 (98%) had a negative blood culture. This indicates that for one proven case of EOS 42 infants were treated (Table 4). The number of infants treated for one case of EOS was 68 in the group infants with a gestational age of 24-27 weeks compared to 21 in infants with a gestational age between 37-42 weeks.

Table 1. Characteristics of inborn infants.

	No.of infants [N=662 (%)]
Male	348 (53)
Gestational Age	
< 28 weeks	75 (11)
28-32 weeks	304 (46)
33-36 weeks	98 (15)
37-42 weeks	185 (28)
Birth weight, n	
< 1500 gram	261 (39)
1500-2499 gram	203 (31)
2500-4500 gram	186 (28)
> 4500 gram	12 (2)
Age of mother (year), mean ± SD	31.0 ± 5.3

The laboratory parameters IT ratio and neutrophilis were predictive for an EOS (Table 5). The positive likelihood ratios of an IT ratio > 0.20 in the first three hours after birth and between 3 and 48 hours were 11 and 18, respectively (Table 6). The negative likelihood ratios for both periods were 0.35. Other laboratory parameters like hemoglobin, leucocyte count, thrombocytes, glucose, pH, standard base excess, umbilical vein or artery pH were not associated with EOS.

Table 2. Characteristics of the mothers and infants suspected for EOS

	Suspected EOS [N=467]	Proven EOS [N=10]	OR	CI 95%	p value
Rupture of membranes > 24h	126	3	1.2	0.3 – 4.6	0.734
Antibiotics During delivery	93	2	1.0	0.2 – 4.8	1.000
Corticosteroid Effective dose	324	5	0.4	0.1 – 1.5	0.183
Vaginal delivery	275	5	0.7	0.2 – 2.4	0.747
Caesarean section	192	5	1.4	0.4 – 5.1	0.747
Maternal fever	41	2	2.7	0.6 – 13.1	0.216
Meconium stained amniotic fluid	56	2	1.9	0.4 – 9.0	0.342
Male	254	7	2.0	0.5 – 7.8	0.358
Gestational Age					
< 28 weeks	70	1	0.6	0.1 – 5.0	1.000
28-32 weeks	258	5	0.8	0.2 – 2.8	0.758
33-36 weeks	56	1	0.8	0.1 – 6.5	1.000
37-42 weeks	83	3	2.0	0.5 – 8.0	0.393
Birth weight					
< 1500 gram	215	5	1.2	0.3 – 4.1	1.000
1500-2499 gram	153	2	0.5	0.1 – 2.4	0.510
2500-4500 gram	93	3	1.8	0.4 – 6.9	0.425
> 4500 gram	6	0	—		
Intrauterine growth					
Small for gestational age (<P2,3)	6	0	—		
Appropriate for gestational age	448	10	—		
Large for gestational age (>P97,7)	13	0	—		
Low Apgar score after 5 minutes					
4-6	52	1	0.9	0.1 – 7.0	1.000
1-3	12	0	—		
Mortality in first week of life	16	1	3.3	0.4 – 27.5	0.297

CI, confidence interval; NS, not significant.

Table 3. Distribution of isolated pathogens among 10 cases of Early Onset Sepsis

Organism	No.
Gram-negative organisms	2
Escherichia coli	1
*Moraxella catarrhalis**	1
Gram-positive organisms	
CO. Negative Staphylococcus	9
Group B Streptococcus	3
Enterococcus faecalis	2
Listeria monocytogenes	1
Streptococcus anginosus	1
*Streptococcus mitis**	1
	1

* One case of early onset sepsis with 2 isolated organisms.

Table 5. Laboratory parameters of the infant and the risk of early onset sepsis

Test	Without EOS	Proven EOS	OR	CI 95%	P value
Abnormal Hb for GA[a] in first 48hrs	37 / 425	0 / 8	—		
CRP > 10 mg / l first 3hours	9 / 96	1 / 3	4.8	0.4-58	NS
CRP > 10 mg / l in 3 – 48 hours	56 / 170	4 / 6	4.1	0.7-22	NS
WBC < 4 or > 24 x 10^9 / l first 48hrs	43 / 433	2 / 6	3.0	0.6 – 15	NS
Neutrophil < 0.7 or > 17 x 10^9 / l first 48hrs	19 / 329	2 / 6	8.2	1.4 – 47	0.048
Thrombocytes < 50 or > 400 x 10^9/l) first 48hrs	23 / 426	0 / 7	—		
IT ratio > 0.20 in first 3 hours post partum	10 / 166	2 / 3	31	2.6 – 374	0.013
IT ratio > 0.20 3 to 48 hours post partum	8 / 214	4 / 6	51	8.2 – 323	< 0.001
Blood Glucose					
< 2.3 mmol / l in first 3hours	78 / 452	2 / 9	1.4	0.3 – 6	NS
< 2.6 mmol / l 3 to 48 hours post partum	162 / 591	1 / 9	0.3	0.04 – 2	NS
pH : < 7.1 in first 3 hours post partum	26 / 314	1 / 5	2.8	0.3 – 25	NS
pH: < 7.2 in 3 to 48 hours post partum	60 / 449	3 / 7	4.9	1.1 – 22	NS
BE < - 10.0 in first 3 hours post partum	37 / 313	1 / 5	1.9	0.2 – 17	NS
BE < - 10.0 in 3 to 48 hours post partum	21 / 449	0 / 7	—		
Umbilical vein pH < 7.11	29 / 471	0 / 9	—		
Umbilical artery pH < 7.11	60 / 512	2 / 9	2.2	0.4-10	NS

[a]GA: gestational age: 24-27wks: 6.4-11.1 mmol/l; 28-32wks: 7.0-12.6 mmol/l ; 33-36wks: 7.0-13.0 mmol/l ; 37-42wks:7.4-13.2 mmol/l

Table 4. Incidence of early onset sepsis and antibiotic therapy for suspected Early onset sepsis (EOS)

	24-27 [N=73]	28-32 [N=306]	33-36 [N=98]	37-42 [N=185]	Total [N=662]
Suspected EOS	70 (96)	258 (84)	56 (57)	83 (45)	467 (71)
Antibiotics given	68 (93)	246 (80)	46 (47)	63 (34)	423 (64)
Cases of EOS	1 (1.4)	5 (1.6)	1 (1.0)	3 (1.6)	10 (1.5)
No.treated for one EOS	68	49	46	21	42

Table 6. Performance characteristics of tests for early onset sepsis

	No.of infants	A priori	Sensitivity	Specificity	PPV	NPV	PLR	NLR
CRP > 10 mg/l in first 3hours	99	3 / 99	33%	90%	0.10	0.98	3.56	0.74
CRP > 10 mg/l in 3 to 48hrs post partum	176	6 / 176	67%	67%	0.07	0.98	2.02	0.50
Leucocytes<4 or > 24 x 10^9 / l first 8hrs	439	6 / 439	33%	90%	0.04	0.99	3.36	0.74
Neutrophil < 0.7 or > 17x10^9 / l) first 8hrs	335	6 / 335	33%	94%	0.10	0.99	5.77	0.71
IT ratio > 0.20 in first 3 hours post partum	169	3 / 169	67%	94%	0.17	0.99	11.0	0.35
IT ratio > 0.20 3 to 48 hours post partum	220	6 / 220	67%	96%	0.33	0.99	17.8	0.35
pH < 7.1 in first 3 hours post partum	319	5 / 319	20%	20%	0.04	0.99	2.42	0.87
pH < 7.2 in 3 to 48 hours post partum	456	7 / 456	43%	43%	0.05	0.99	3.21	0.66

Discussion

We found a low incidence of blood culture-proven EOS. The incidence in our NICU was 1.5/100 inborn infants who were admitted the NICU on the

first 2 days of life. The incidence in our analyses of EOS in premature infants is representative for the situation in Western countries (2;5). The incidence of EOS in preterm and term infants admitted to this NICU was not different. Despite the low incidence of EOS, it was often suspected: 71% of all admitted newborn infants were suspected for having EOS, and 91% of these infants received antibiotics. The rate of prescription of antibiotics for suspected EOS versus blood culture proven EOS was high. For each case of proven EOS many infants were treated with antibiotics. The problem of unnecessary antibiotic treatment was most pronounced in infants born with a very low gestational age, but the "overuse" of antibiotics was not only restricted to these infants. In term infants for each case of proven EOS 21 infants were treated with antibiotics.

We used the blood culture as golden standard for detecting EOS. Neonates with sepsis have often a high-colony-count bacteriaemia and most studies suggest that the sensitivity of a blood culture is 90% or slightly more (8; 13-15). When mothers received intrapartum antibiotics the sensitivity of the blood culture decreased (16). Antibiotics were given during delivery to the mothers of 16% of the infants in the analysis. When taking this number in account we assume that we might have missed approximately two cases of EOS when the mother received intrapartum antibiotics and one other case of EOS in the group were mothers did not receive intrapartum antibiotics. This does not influence our conclusions that too much preterm and term infants receive antibiotics in their first days after birth.

The restricted use of intrapartum antibiotics in the study is related to a protocol for intrapartum antibiotic treatment to prescribe antibiotics only when risk factors are present. A vaginal swap is done in pregnancy when one or more risk factors for a neonatal Group B Streptococcal (GBS) sepsis are present (17). These risk factors are PROM (>18 hours), premature delivery, maternal fever, urinary tract infection or mothers who gave birth to an infant with GBS-sepsis in a previous pregnancy. Amoxicillin is given at least 4 hours before delivery when the vaginal swap is positive for a group B *streptococcus*,. Recent reports suggest that the intrapartum use of antibiotics reduced the prevalence of early onset GBS sepsis and increased the incidence of early onset *E.coli* sepsis. We noted a low

incidence of early onset *E.coli* sepsis. Early onset GBS sepsis occurred in only 2 infants (20%).

The reasons for the wide spread use of antibiotics in case of suspected EOS are 1) clinical signs are non-spesific and can occur in the absence of an infection, 2) maternal risk factors raise the suspicion for EOS but only a few infants whose mothers had risk factors developed an EOS, and 3) ruling out EOS with common diagnostic tests is difficult. The used diagnostic tools, such as C-reactive protein, leucocyte count and IT ratio have a low sensitivity in the early phase of an infection (18;19). Infants with a negative test can have EOS. A blood culture is definite negative after 36-72 hours and therefore not available for clinicians in the decision to initiate antibiotic treatment or not.

Early diagnosis and treatment of the newborn infant with antibiotics for suspected EOS is essential to prevent severe and life threatening complications. But unnecessary treatment with antibiotics has to be avoided, because the use of broad spectrum antibiotics in newborn has several side effects for the individual infant and the whole NICU population. Gut colonization in the infants changes, overgrowth of fungal and Gram-negative micro-organisms might occur (9-11). These changes in colonization make newborns more susceptible for Gram-negative and fungal infections (9;20). Broad-spectrum antibiotics might select resistant micro-organisms in the NICU (9). Decreasing the unnecessary use of antibiotics in preterm and term infants could reduce the incidence of late onset bacterial and fungal sepsis and reduce the rates of outbreaks with multi resistant bacteria.

Since the 90s promising studies with cytokines and cell surface markers are published. None of the reports show 100% sensitivity (21). A multi=center randomized intervention study reported that with an IL-8 measurement directly after birth the use of antibiotics for EOS was reduced from 50% to 36% in infants suspected for EOS (22). Other reports showed a higher sensitivity and specificity for cytokines than the commonly used diagnostic tests (23-25). But the relatively small sample size in most studies and different cut off points of the same diagnostic marker given in the literature makes it difficult to interpret the results and to start using the diagnostic tool clinically.

In conclusion, EOS is often suspected and newborn infants are frequently treated with antibiotics. With an incidence of 1,5/ 100 admitted infants, EOS is uncommon. For each case of EOS,42 infants are treated with antibiotics. This problem of unnecessary antibiotics treatment is most severe in infants born with a very low gestational age. But the “overuse” of antibiotics is not only restricted to infants born with a very low gestational age. More studies are needed to find a robust diagnostic test that can definitely rule out EOS in term and preterm infants before antibiotic treatment is started.

References

1. Bizzaro MJ, Raskind C, Baltimore RS, Gallagher PG. Seventy-five years of neonatal sepsis at Yale: 1928-2003. Pediatrics 2005;116(3):595-602.

2. Lopez Sastre JB, Coto Cotallo GD, Fernandez CB. Neonatal sepsis of vertical transmission: an epidemiological study from the "Grupo de Hospitales Castrillo". J Perinat Med 2000;28(4):309-15.

3. Ronnestad A, Abrahamsen TG, Medbo S, Registad H, Lossius K, Kaaresen PI, et al. Septicemia in the first week of life in a Norwegian national cohort of extremely premature infants. Pediatrics 2005;115(3):e262-e268.

4. Stoll BJ, Gordon T, Korones SB, Shankaran S, Tyson JE, Bauer CR, et al. Early-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr 1996;129(1):72-80.

5. Stoll BJ, Hansen NI, Higgins RD, Fanaroff AA, Duara S, Goldberg R, et al. Very low birth weight preterm infants with early onset neonatal sepsis: the predominance of Gram-negative infections continues in the National Institute of Child Health and Human Development Neonatal Research Network, 2002-2003. Pediatr Infect Dis J 2005;24(7):635-9.

6. Isaacs D, Wilkinson AR, Moxon ER. Duration of antibiotic courses for neonates. Arch Dis Child 1987;62(7):727-8.

7. Kaiser JR, Cassat JE, Lewno MJ. Should antibiotics be discontinued at 48 hours for negative late-onset sepsis evaluations in the neonatal intensive care unit? J Perinatol 2002;22(6):445-7.

8. Solorzano-Santos F, Miranda-Novales MG, Leanos-Miranda B, az-Ponce H, Palacios-Saucedo G. A blood micro-culture system for the diagnosis of bacteremia in pediatric patients. Scand J Infect Dis 1998;30(5):481-3.

9. de Man P, Verhoeven BA, Verburgh HA, Vos MC, van de Anker JN. An antibiotic policy to prevent emergence of resistant bacilli. Lancet 200018;355(9208):973-8.

10. Fanaro S, Chierici R, Guerrini P, Vigi V. Intestinal microflora in early infancy: composition and development. Acta paediatr Suppl 2003;91(441):48-55.

11. Saiman L, Ludington E, Pfaller M, Rangel-Frausto S, Wilbin RT, Dawson J, et al. Risk factors for candidemia in Neonatal Intensive Care Unit patients. The National Epidemiology of Mycosis Survey study group. Pediatr Infect Dis J 2000;19(4):319-24.

12. Kollee LA, den Ouden AL, Drewes JG, Brouwers HA, Verwey RA, Verloove-Vanhorick SP. [Increase in perinatal referral to regional centers of premature birth in The Netherlands: comparison 1983 and 1993]. Ned Tijdschr Geneeskd 1998;142(3):131-4.

13. Remington, Klein. Infectious diseases of the fetus and newborn infant. 6 ed. 2006.

14. Shelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP. Volume of blood required to detect common neonatal pathogens. J Pediatr 1996;129(2):275-8.

15. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteremia and fungemia. Clin Microbiol Rev 1997;10(3):444-65.

16. Hsu KK, Pelton SI, Shapiro DS. Detection of group B streptococcal bacteremia in stimulated intrapartum antimicrobial prophylaxis. Diagn Microbiol infect Dis 2003;45(1)23-7.

17. ACOG Committee Opinion: number 279, December 2002. Prevention of early-onset group B streptococcal disease in newborns. Obstet Gynecol 2002;100(6):1405-12.

18. Benitz WE, Han MY, Madan A, Ramachandra P. Serial serum C-reactive protein levels in the diagnosis of neonatal infection. Pediatrics 1998;102(4):E41.

19. Fowli PW, Schmidt B. Diagnostic tests for bacterial infection from birth to 90 days-a systematic review. Arch Dis Child Fetal Neonatal Ed 1998;78(2):F92-F98.

20. Weese-Mayer DE, Fondriest DW, Brouillette RT, Shulman ST. Risk factors associated with candidemia in the neonatal intensive care unit: a case-control study. Pediatr Infect Dis J 1987;6(2):190-6.

21. Malik A, Hui CP, Pennie RA, Kirpalani H. Beyond the complete blood cell count and C-reactive protein: a systematic review of modern diagnostic tests for neonatal sepsis. Arch Pediatr Adolesc Med 2003;157(6):511-6.

22. Franz AR, Bauer K, Schalk a, Garland SM, Bowman ED, Rex K, et al. Measurement of interleukin 8 in combination with C-reactive protein reduced unnecessary antibiotic therapy in newborn infants; a multicenter, randomized, controlled trial. Pediatrics 2004;114(1):1-8.

23. Franz AR, Steinbach G, Kron M, Pohlandt F. Interleukin-8: a valuable tool to restrict antibiotic therapy in newborn infants. Acta Paediatr 2001;90(9):1025-32.

24. Nupponen I, Andersson S, Jarvenpaa AL, Kautiainen H, repo H. Neutrophil CD11b expression and circulating interleukin-8 as diagnostic markers for early-onset neonatal sepsis. Pediatrics 2001;108(1):E12.

25. Resch B, Gusenleitner W, Muller WD. Procalcitonin and interleukin-6 in the diagnosis of early-onset sepsis of the neonate. Acta Paediatr 2003;92(2):243-5.

CHAPTER 6

Levels of 25 cytokines in the first seven days of life in newborn infants

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Abstract

Backgrounds. Novel methods for cytokine analysis allow for the simultaneous measurement of 25 cytokines in 50µL serum or plasma. Data on normal values of most of these cytokines in healthy preterm and term newborn infants are lacking.

Objective. To analyze levels of 25 cytokines in the first week of life in healthy preterm and term infants and relate them to gestational age.

Patients and Methods. Thirty-four healthy inborn-infants were selected from a prospective study conducted between October-2007 till October-2009 in NICU of Harapan Kita Women and Children's Hospital Jakarta-Indonesia. Twenty-five cytokines were measured. These infants were grouped according to GA: 30-32wks(n=11), 33-35wks(n=14) and ≥36wks(n=9). All infants were stable during the first week and had a negative blood culture on admission.

Results. Only MIG was lower at birth in infants 30-32wks compared to the other groups. Except for IL-1Ra and IL-6, where higher values were found during the first four hours after birth, no trend over time was found in any of the cytokines. Between 24 and 72hrs levels of IL-1Ra, IL-2, IL-8, IL-12, IL-13, IL-15, IL-17, IFNγ, MIP-1a, MCP-1, TNFα were lower in infants 30-32wks compared to infants ≥36wks; levels of IL-6, IL-10, IP-10 were lower in both preterm groups. No difference between groups for any of the levels was found for IL-1b, IL-2r, IL-4, IL-5, IL-7, IFNa, MIP-1b, GM-CSF, Eotaxin and RANTES.

Conclusions. Levels of 25 interleukines are stable in the first week of life in non-infected infants. Infants born 30-32wks showed lower levels of IL-1Ra, IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IP-10, IFNγ, MIP-1a, MCP-1, TNFα compared to infants born ≥36wks after 72 hrs. This indicates a lower stimulation or activation of Th-1 cells, monocytes and dendritic cells in these infants. Our data can be used as normal values in future studies evaluating their use in diagnosing infections in newborn infants.

Key words: term-newborn,preterm-infants ,cytokines,chemokines

Abbreviations :NICU,neonatal Intensive care unit; IL,interleukin; AS,Apgar Score; TNFα,Tumor necrosis factor alpha; IFNγ,Interferon gamma; CRP,C-reactive proteine; TH,T-helper; MCP1,monocyte chemoattaractant proteine-1; MIP1a/b, Macrophage inflammatory proteine-1a/b

Introduction

Infections are an important cause of morbidity and mortality in preterm as well as term infants[1]. Clinical signs that might indicate the presence of an infection are not specific. Antibiotics are used frequently when an infection is suspected. The widespread use of antibiotics has a number of important side-effects, including the occurrence of bacterial resistance and the development of an abnormal gastro-intestinal flora[2]. Reliable methods to detect an infection in newborn infants therefore are needed. Cytokines and chemokines have been evaluated for this purpose. However, they are still not used in clinical practice. Studies found higher levels of IL-1Ra,IL-6,IL-8,IP-10 and MIP-1a in infants with a proven infection, but there was overlap in results between infants with and without an infection [3-11]. Data on cytokine levels in the first week of life in healthy, non-infected infants are very limited[12-14]. For some of the cytokines and chemokines an increase after birth was found, while for others a decrease was observed [15,16]. Another unresolved question is the potential effect of gestational age on cytokine levels. In most studies only preterm infants are included.

Studies conducted so far included a limited number of interleukines. Using the Luminex array it has become possible to measure up to 25 cytokines and chemokines at the same time in only 50 µl of plasma or serum. A number of these 25 cytokines have not been evaluated yet as potential markers for neonatal infection. Before a cytokine can be used in daily practice it is essential to know levels in non infected infants and to know if these level are related to gestational age. The aim of this study is to measure sequentially in the first week of life 25 cytokines and chemokines in infants with different gestational ages admitted to our neonatal intensive care unit.

Research methods

Subjects: This study is part of a large prospective study on interleukin levels conducted at the NICU of Harapan-Kita Women and Children Hospital, Jakarta, Indonesia from October-2007 till October-2009. For the present study patients were selected when they were inborn and showed

no clinical signs compatible with an infection during the first seven days of life other than mild respiratory problems for which N-CPAP was given with 21% of FiO_2. As CPAP is used very frequent in our unit for mild respiratory problems in infants without other symptoms, the use of CPAP was allowed. The blood culture, taken soon after birth, had to be negative. As is practice in our NICU, all infants admitted with -mild- respiratory problem, were treated with broad spectrum antibiotics (Ampicillin-sulbactan and an aminoglycoside). The study was approved by the Research Ethical Committee, Harapan Kita Women and Children Hospital, Jakarta.

Routine laboratory and cytokine measurement: Blood was taken on admission for clinical purposes, including a blood culture. An additional 0,3 ml was taken for cytokine levels. Thereafter the same amount of blood was taken at 4,12,24 hrs and at day 2,3,4,5,6 and 7 together with blood taking for clinical purposes. In infants with a gestational age of 32 weeks and less the blood sampling for this study was stopped after day three, as is was considered not ethical to take too much blood in these tiny infants for study purposes. Immediately the blood sample was centrifuged, 50 μl of serum was sampled and stored at -80°C. Samples were shipped on dry ice to the University Medical Center Groningen, The Netherlands, where they were analyzed. Sera were thawed and analysed using Invitrogen's Multiplex Bead Immunoassay. In a 96 well plate samples were prepared by adding beads of defined spectral properties which were conjugated to protein-specific capture antibodies, incubation buffer to bind cytokines to the protein-specific capture antibodies and biotinylated detector antibodies. Finally streptavidin conjugated to the light-sensitive fluorescent protein R-Phycoerythrin was added and cytokine concentrations were analyzed with the Luminex detection system (Luminex Corp., Austin, Texas) using the programme StarStation 2.3. By monitoring the spectral properties of the beads and the amount of associated R-Phycoerythrin (RPE) fluorescence, the concentration of proteins was determined. The cytokines/chemokines IL-1b,IL-1Ra,IL-2,IL-2r,IL-4,IL-5,IL-6,IL-7,IL-8(CXCL-8),IL-10,IL-12,IL-13,IL-15,IL-17,TNFα,IFNa,IFNγ,IP-10(CXCL-10),MIP-1a(CCL-3),MIP-1b(CCL-4),Eotaxin,Rantes(CCL-5),GM-CSF,MIG,MCP-1 (CCL-2) were measured.

Statistical analysis: To study the effect of gestational age the infants were divided in three groups, 30-32wks, 33-35wks and ≥36 wks. Differences

between groups were analyzed by Non Parametric statistic (Mann-Whitney U test). The trend over time was evaluated using the Rank- Spearman test. To correct for multiple comparisons, the significance for all analysis was set at $p<0.01$.

Results

During the two-years period of our prospective study we selected thirty-four healthy inborn-infants admitted at our NICU of Harapan Kita Women and Children's Hospital. Clinical characteristics of the infants are given in Table 1. None of the infants did have perinatal asphyxia, as indicated by an Apgar score of at least 5 after 5 minutes. The majority of infants were born by caesarean section. One mother showed fever before birth. As is practice in our hospital, the majority of mothers received antibiotics just before birth, mainly because of the caesarean section. The blood culture was negative in all infants, except for one preterm infant who showed a positive blood culture with Coagulase positive staphylococci, this was considered a contamination.

All cytokines and chemokines were above the detection limit in almost all infants. The results of all interleukin levels in the three groups are presented in table 2. There was no in cytokines levels between who received antibiotics for less and more than five days. IL-1Ra and IL-6 showed higher and more variable values in the first four hours after birth compared to the period there after, no trend over time was found during the rest of the first week In all three groups no trend over time was found in any of the other interleukines. Levels of IL-1b,IL-2r,IL-4,IL-5,IL-7,IFNa,GM-CSF,MIP-1b,Eotaxin and RANTES were not different at any time point between groups. Levels of IL-2,IL-15,IL-17 and TNFα were lower in the infants born at 30-32wks compared to the group > 36wks at 4 and 24-72 hours. In the period 24-72 hours, levels of IL-1Ra,IL-6,IL-8,IL-12,IL-13,IFNγ,MIP-1a,IP-10 and MCP-1 were lower in the group 30- 32wks compared to ≥36wks. Levels of IL-6,IL-10 and IP-10 were lower in the period 24-72 hours in the group 30-32 wks compared to the older infants. MIG was lower at birth in infants 30-32 weeks compared to the other groups.

Table1. Characteristics of study groups

Characteristics	≥ 36 weeks (n=9)	33–35 weeks (n=14)	30-32 weeks (n = 11)
GA (wks), median (25-75 th)	38 (36,5 – 38,5)	34 (33 – 34)	32 (31 – 32)
BW (gram), mean (range)	2586 (1800 – 3232)	1871 (1671 – 2091)	1557 (1415 – 1662)
C-Section	9	10	10
Gender: Male	6	7	3
AS less than 5 at 5 min	0	0	0
Clinical Amnionitis[@]	2	2	4
CPAP (duration (days))	8 (3)	11 (3)	10 (3)
Leucocyte < 5000 or > 30.000/mm3	2	0	1
Thrombocyte < 150.000 or > 600.000/mm3	2	3	1
Day antibiotics (median)	4 (4 – 6)	4 (4 – 7)	5 (3 – 12)
Length of hospitalization at NICU (mean)(days)	8 (6 – 9)	7 (4 – 10)	16 (8 – 23)

@ : PROM more than 12 hours; decceleration CTG; Maternal fever ≥ 38'C; Maternal leucocytes > 15.000/mm^3

Table2. Serum concentration of 25 cytokines between study groups during first week of life. Data shown as median (min-max)

Cytokines			≤ 32 wks		33 - 35 wks		≥ 36 wks	
IL-1b	-	0	0	(0-15)	1	(0-23)	7	(0-17)
	-	4	0	(0-4)	1	(0-30)	4	(0-20)
	-	24-72	0	(0-1)	1	(0-8)	4	(0-23)
	-	96-168			4	(0-17)	4	(0-14)
IL-1Ra	-	0	413	(158-931)	512	(179-22246)	803	(200-2376)
	-	4	2026	(63-13795)	596	(119-10464)	4183	(331-14702)
	-	24-72	242	(168-354)[@&]	508,5	(737-302,5)	545	(407-698)
	-	96-168			395,5	(310-557)	441	(179-613)
IL-2	-	0	3,5	(3-7)	4	(1-15)	14	(3-18)
	-	4	3	(3-5)[@]	4	(1-17)	14	(4-17)
	-	24-72	3.5	(3-6.75)[@]	4	(3-11)	14	(5-15)
	-	96-168			9,5	(3-16)	14	(13-16)
IL-2r	-	0	325	(236-427)	365	(202-604)	295	(145-507)
	-	4	300	(219-443)	349	(179-621)	295	(212-604)
	-	24-72	458	(332-617)	427	(268-556)	507	(387-631)
	-	96-168			692	(356-846)	610	(572-652)
IL-4	-	0	2	(0-5)	5	(2-112)	2	(1-3)
	-	4	2	(0-5)	5	(2-128)	5	(1-10)
	-	24-72	2	(0-2)	4	(2-94)	3	(1-5,5)
	-	96-168			4,5	(2-22)	3	(1-6)

		≤ 32 wks		33 - 35 wks		≥ 36 wks	
IL-6 -	0	2	(0-2)	5	(0-34)	6	(0-8)
-	4	2	(0-3)	5	(0-33)	6	(0-8)
-	24-72	2,5	(2-4)	5	(2-7)	6	(2-8)
-	96-168			8,5	(6-10)	6	(6-7)
IL-8-	0	15	(0-1440)	30,5	(1-418)	79	(64-232)
-	4	12	(1-251)	26	(1-212)	94	(7-3135)
-	24-72	4	(2-11)@	5,5	(2-16)@@	17	(3-38)
-	96-168			9,5	(5-29)	3	(2-8)
IL-7-	0	0	(0-0)	6	(0-40)	0,5	(0-1)
-	4	0	(0-12)	3	(0-32)	0,5	(0-3)
-	24-72	0	(0-0)	0	(0-8)	0	(0-6)
-	96-168			3,5	(0-13,5)	0	(0-5)
IL-8-	0	57	(8-204)	98,5	(1-396)	40	(34-46)
-	4	28,5	(10-157)	46	(1-189)	82,5	(13-189)
-	24-72	19,5	(13-40)@&	44	(23-86)	57	(28-170)
-	96-168			44,5	(24-78)	37,5	(13-64)
IL-10-	0	3	(3-29)	14,5	(0-74)	22	(22-25)
-	4	3,5	(1-28)	9	(1-49)	23	(2-48)
-	24-72	3	(2-3)@	3	(2-11)@	21	(3-22)
-	96-168			14	(3-21)	21	(3-21)

		≤ 32 wks		33 - 35 wks		≥ 36 wks	
IFNa-	0	36	(20-49)	40	(18-88)	30	(8-58)
-	4	20	(0-49)	36	(18-98)	49	(20-79)
-	24-72	36	(23-49)	49	(26-65)	36	(30-45)
-	96-168			44,5	(36-75)	40	(30-49)
IFNγ-	0	3	(3-3)	8	(2-449)	7	(3-8)
-	4	3	(2-4)	8	(3-603)	7	(3-9)
-	24-72	3	(3-4)[@&]	7,5	(3-43)	7	(4-8)
-	96-168			8	(4-16)	7	(7-8)
GM-CSF							
-	0	0	(0-0)	1	(0-48)	0	(0-1)
-	4	0	(0-0)	1	(0-27)	0	(0-34)
-	24-72	0	(0-0)	0	(0-2)	0	(0-10)
-	96-168			1	(0-22)	0	(0-7)
MIP-1a-	0	24	(22-32)	28	(23-150)	28	(19-34)
-	4	23	(22-34)	26	(18-150)	30	(23-36)
-	24-72	23,5	(23-25)[@&]	29,5	(25-37)	28	(26-37)
-	96-168			30	(25-38)	26	(24-32)
MIP-1b-	0	82	(23-191)	74	(34-345)	40	(22-237)
-	4	74	(51-133)	66	(41-550)	83	(57-250)
-	24-72	41,5	(21-110)	80	(54-145)	66	(47-120)
-	96-168			86,5	(45-149)	60	(42-90)

		≤ 32 wks		33 - 35 wks		≥ 36 wks	
IL-12-	0	418,5	(179-693)	473	(215-815)	278	(202-1837)
-	4	461,5	(144-628)	345	(171-960)	1151	(258-1883)
-	24-72	263	(225-339)[@&]	384	(200-518)	402	(296-499)
-	96-168			358	(244-446)	431	(219-602)
IL-13-	0	0	(0-7)	7	(0-26)	12	(0-70)
-	4	0	(0-7)	1	(0-22)	14,5	(0-81)
-	24-72	0	(0-7)[@]	1,5	(0-8,5)	12	(0-25)
-	96-168			7	(0-17)	17	(12-26)
IL-15-	0	0	(0-0)	13	(0-64)	37	(0-49)
-	4	0	(0-0)[@&]	10	(0-49)	39	(0-53)
-	24-72	0	(0-0)[@&]	13	(0-42)	41	(0-53)
-	96-168			39,5	(0-52)	37	(28-47)
IL-17-	0	0	(0-0)	36	(0-130)	23	(0-33)
-	4	0	(0-6)[@&]	26	(0-146)	22	(6-38)
-	24-72	0	(0-1)[@&]	27,5	(1-45)	18	(8-31)
-	96-168			29	(5-42)	19	(0-28)
TNFα	0	0	(0-0)	1	(0-6)	3	(0-6)
-	4	0	(0-0)[@&]	1	(0-7)	3	(0-5)
-	24-72	0	(0-0)[@&]	1	(0-4)	3	(0-5)
-	96-168			4	(0-6)	4	(0-4)

		≤ 32 wks		33 - 35 wks		≥ 36 wks	
IP-10-	0	19	(8-144)	32,5	(8-85)	115	(16-124)
-	4	29,5	(3-162)	29	(6-119)	72,5	(19-119)
-	24-72	24,5	(16-54)@	37	(19-56)	82,5	(47-137)
-	96-168			61	(39-140)	55,5	(34-134)
MIG-	0	32	(23-71)@	41	(8-118)	0	(0-8)
-	4	23	(0-41)	23	(0-145)	8	(0-47)
-	24-72	23	(23-41)	35,5	(23-57)	41	(2-47)
-	96-168			61	(39-140)	23	(8-47)
Eotaxin-	0	24	(14-37)	35	(10-221)	9	(4-40)
-	4	30,5	(11-80)	33	(10-86)	39	(12-220)
-	24-72	30	(16-65)	51	(36-82)	32,5	(26-62)
-	96-168			54	(28-67)	41	(25-55)
Rantes-	0	1200	(1200-4967)	1200	(1000-1828)	1000	(1000-1200)
-	4	1200	(1200-9900)	1200	(1000-1824)	1000	(1000-1443)
-	24-72	1200	(1200-2821)	1200	(1200-1068)	1000	(1000-1200)
-	96-168			1100	(1000-1718)	1000	(1000-1132)
MCP-1-	0	440	(234-3238)	399	(120-2252)	163	(69-2786)
-	4	355	(0-3841)	433	(84-1685)	1119	(210-11314)
-	24-72	275	(141-516)@	463	(320-836)	934	(401-3650)
-	96-168			378	(271-651)	406	(189-879)

Mann Whitney-U test : @ : $p<0.01$; comparison between group ≤32 wks / 33-35 wks and group term

& : $p<0.01$; comparison betweeen group ≤32 wks and group 33-35 wks

Discussion

In this paper we show that levels of 25 cytokines/chemokines in healthy, not infected newborn infants are constant between day 2 and 7 in both preterm and term infants. Levels of IL-1Ra,IL-2,IL-6,IL-8,IL-10,IL-12,IL-13,IL-15,IL-17,TNFα,IFNγ,MIP-1a,IP-10 and MCP-1 are lower in infants born at 30-32wks compared to infants born at or ≥36wks at day two and three days of life. IL-6,IL-10 and IP-10 were also lower in the group born between 33 and 35 weeks compared the group ≥36wks.

Only very few studies evaluated sequentially cytokines in newborn infants. In one of the first papers on the pattern of cytokines Kuster et al[3] described the pattern of IL-1Ra and IL-6 starting 4 days before a clinical sepsis till 5 days thereafter. Sepsis was diagnosed at 16 ± 13 days. Both IL-6 and IL-1Ra increased before the clinical signs of infection, no conclusion as to the normal pattern of these cytokines is possible from this study. Rizos et al[12] measured IL-2,IL-2r,IL-4,IL-4r and IFNγ on day 1 and day 5 in term infants. They found no change in the level of IL-2, and an increase in IL-2r and IFNγ on day 5 compared to day 1. In our study, in term infants we did not find a change in IL-2 and IL-2r during the whole first week of life. We found lower levels of IFNγ during the first 72 hours of life in preterm infants but no trend in term infants during the whole first week. Damman et al[15] described patterns of 16 cytokines/chemokines during the first week in 15 preterm infants born with gestational age of ≤28wks. They found in a number of cytokines, especially pro-inflammatory cytokines, a decreasing level in the first 3 days and a stable pattern thereafter. It is difficult to compare these to our data as Damman et al had no information regarding the clinical condition of the infant. Other studies have shown that higher levels in the first days of life might have been influenced by factors like infection, asphyxia, rescucitation or ventilation[17-19]. All these factors have shown to increase cytokine levels at birth[7,19]. We are convinced that infants included in our study did not have an infection while they neither had signs of asphyxia. The slightly variable levels in our study we observed in the first day compared to the period thereafter for IL-1Ra and IL-6 may be due to the type of delivery, the use of oxygen during rescucitation and undetected chorioamnionitis, not resulting in a neonatal infection. Sullivan et al[13] studied levels of four chemokines

in cord blood in both healthy preterm and term infants. They did not find a difference in levels of MIP-1a,Eotaxin and Rantes between infants born preterm and term. In our study, we found lower levels of MIP-1a in infants of 30-32 wks compared to infants ≥36 wks. An explanation might be that we took blood in infants after birth while Sullivan measured cord blood. Matoba et al[14] recently described levels of 27 immune biomarkers in cord blood. Concentrations of 12 biomarkers (IL-2,IL-4,IL-5,IL-8,IL-10,MCP-1,MIP-1a,MIP-1b,sIL-6ra,sTNF-RI,TNFα and TREM-1) were higher in preterm compared to term infants, while IL-1b and IL-18 were lower. These authors assumed that the higher level of a number of cytokines were related to preterm birth. It is questionable however if preterm birth itself caused an increased level in cytokines or that a common factor like infection caused both an increase in cytokines level and preterm birth. The incidence of chorioamnionitis for instance was higher in group preterm compared to term infants. Levels of the pro-inflammatory cytokines MIP-1a and MIP-1b showed the highest correlation between gestational age and cytokines levels. Preliminary data from studies conducted by us as well as other studies indicate that MIP1a and MIP1b are specific markers for a bacterial infection. The study of Matoba did not provide data on blood cultures in the infants, also no data on cytokine levels during the first week of life. Our results are different from these data of Matoba, an explanation might be that we selected only infants without signs of infection or asphyxia. A recent study by McElrath et al[20] showed that cytokines measured on the first day of life were higher in preterm infants born after complications that are associated with infections compared to preterm infants born after complications like pre-ecclampsia. Blanco-Quiros[21] observed higher values of IL-10, but not of IL-12 in preterm compared to term infants. The higher levels of IL-10 were mainly found in infants less than 30 weeks, not included in our study. Moreover, they analysed cord blood while we took our first sample from the infant itself. Dembinski et al[22] did not find an influence of gestational age on IL-10 levels in cord blood. In that study only IL-6 and GM-CSF were higher in cord blood in term vs. preterm infants. Schultz et al[23] found no difference in cord blood samples of term and preterm infants when they stimulated cells ex vivo to produce IL-10.

The pattern of a number of cytokines measured on day 1,3,7,14 and 21 of life in ELBW infants (BW<1000g, GA<28wks) was described by Schelonka

et al[16]. Almost half of these infants developed a sepsis after that period. They observed a decreasing trend for IFNγ,TNFα,IL-2,IL-17,IL-4,IL-5,IL-6 and IL-10 from day one to day 21. The largest decrease was found between day one and day 7. The levels of cytokines as found by Schelonka et al[16] during the first week of life were much higher compared to levels we found. This might be due to differences in techniques used, but also to a higher stress level in these very tiny infants compared to our groups. We included only infants with no known factors that might have increased cytokine levels. Interestingly, in the study of Schelonka et al[16] both pro- and anti-inflammatory cytokines decreased during the first weeks of life, the balance between pro- and anti-inflammatory cytokines remained constant.

Natarajan[24] recently described levels of MIP-1a,MIP-1b,RANTES and MCP-1 in extremely preterm infants on day 1 and 3 of life and evaluated the effect of the administration of oxygen. No difference in levels of MIP-1a,MIP-1b and RANTES was found between day 1 and 3, while oxygen did not have an effect on the levels. Prolonged oxygen exposure resulted in elevated levels of MCP-1 on day 3. We observed a rather wide range in MCP-1 levels at 0 and 4 hrs, while the level was lower in the preterm infants compared to the infants $\geq$36 wks between 24-72 hours. In our study the infants did not receive supplemental oxygen that can explain the difference in results.

Our results might indicate less stimulation or activity of Th-1 cells, monocytes and dendritic-cells in the more preterm infants. IL-2,TNFα and IFNγ are produced by Th-1 cells, while IL-1Ra,IL-8,IL-15,IP-10 and MCP-1 are produced mainly by monocytes. Finally,IL-6,IL-10,IL-12,MIP-1a and MIP-1b are mainly produced by dendritic cells. All these interleukins were lower in the more preterm infants. In contrast, of the cytokines produced by Th-2 cells, only IL-13 was lower in the preterm infants.

Our study has a number of limitations. The number of infants included into the study is relatively small. It is difficult however to take sequential, daily, blood samples in healthy preterm and term infants. Therefore we included infants who after birth were on N-CPAP that needed to take blood samples. Secondly, we did not include infants of less than 30 weeks. At the start of

the study we realized that the number of these infants admitted to our unit is too small to be able to include a sufficient number in this study.

Flllllllinally, a limitation of our study is that we included infants who did receivlle CPAP and therefore might be not considered as completely healthy infants. There were no other signs that made them suspected of having an infection. All infants included in this study received antibiotics, as it is practice in our unit to prescribe antibiotics to all infants showing respiratory problems at birth. Still, all infants were stable during the whole first week of life, not showing signs indicating an infection. In all infants a blood culture was taken on admission and found to be negative. Our definition of the non infected group is consistent with the definition used by Ng et al[9]

In conclusion, to our knowledge for the first time, we measured 25 cytokines/chemokines in the first week of life in preterm and term infants without an infection. Patterns were stable after the first 24-48 hrs of life for all cytokines. Preterm infants born at 30-32 wks showed lower levels of fourteen of the 25 measured cytokines compared to term infants. This might indicate a lower stimulation of Th-1 cells, monocytes and dendritic-cells in these infants. Our data can be used as reference data in future studies.

References

1. Stoll BJ, Hansen N, Fanaroff AA et al. Late onset sepsis in very low birth weight neonates: The experience of the NICHD Neonatal Research Network. Pediatrics, 2002; 110(2): 285-91

2. de Man P, Verhoeven BA, Verbrugh HA et al. An antibiotic policy to prevent emergence of resistant bacilli .Lancet, 2000; 355(9208):973-8.

3. Kuster H, Weiss M, Willeitner AE et al. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestations. Lancet, 1998; 352(9136): 1271-7

4. De Bont, Martens A, van Raan S et al. Tumor Necrosis factor alpha, interleukin-1B and interleukin-6 Plasma Levels in Neonatal Sepsis, Pediatric Res, 1992; 380-3

5. Maciolek VM, Thijsen SF, Hemels M et al. Inflammatory mediators for the Diagnosis
and Treatment of Sepsis in Early Infancy. Pediatric Res, 2006; 59: 457-61

6. Hodge G, Hodge S, Haslam R et al. Rapid simultaneous measurement of multiple cytokines using 100uL sample volume-association with neonatal sepsis. Clin Exp Immunol, 2004; 137: 402-7

7. Fotopoulos S, Pavlou K, Skouteli H et al. Early markers of brain damage in premature low birth-weight neonates who suffered from perinatal asphyxia and/or infection. Biol Neonate, 2001; 79(3-4): 213-8

8. Ng PC, Li I, Leu TF et al. Early prediction sepsis induced disseminated intravascular coagulation with interleukin-10,

interleukin-6 and RANTES in preterm infants. Clin Chem, 2006; 52(6): 1181-9

9. Ng PC, Li K, Chui KM et al. IP-10 is an Early Diagnostic Marker for Identification of Late Onset Bacterial Infection in Preterm Infants. Pediatric Res, 2007; 61: 93-8

10. Bender L, Thaarup J, Varming K et al. Early and late markers for the detection of early-onset neonatal sepsis. Danish Medical Bulletin, 2008; 55: 219-23

11. Cancelier AC, Petronilho F, Reinke A et .al. Inflamatory and oxidative parameters in cord blood as diagnostic of early-onset neonatal sepsis: A case control study. Pediatr Crit Care Med, 2009; 10(4): 467-71

12. Rizos D, Protonotariou E, Malamitsi A et al. Cytokine concentration during the first days of life. European J Obstetric and Gynecology and Reproductive Biology, 2007; 131: 32-5

13. Sullivan SE, Staba SL, Gersting JA et al. Circulating concentrations of chemokine in cord blood, neonates and adults. Pediatr Res, 2001; 51(5): 653-7

14. Matoba N, Yu Y, Mestan K et al. Differential patterns of 27 cord blood immune biomarkers across gestational age. Pediatrics, 2009; 123(5): 1320-8

15. Damman O, Leviton A. Matenal Intrauterine Infection, Cytokine and Brain damage in the preterm newborn. Pediatr Res, 1997 ;42(1):1-8.

16. Schelonka RL, Maheshwari A, Carlo WA et al. T cell cytokines and the risk of blood stream infection in extremely low birth weight infants. Cytokine, 2010: December: 1-7

17. Xanthou M, Fotopoulos S, Mouchtouri A et al. Inflammatory mediators in Perinatal asphyxia and infection. Acta Pediatr Suppl. ,2002; 91(438): 92-7

18. Kroon AA, Wang J, Huang Z et al. Inflammatory response to oxygen and endotoxin in newborn rat lung ventilated with low tidal volume. Pediatr Res, 2010;68(1):63-9.

19. Bohrer B, Silveira R, Neto EC et al. Mechanical Ventilation of Newborn Infant Changes in Plasma Pro and Anti Inflammatory Cytokines. The Journal of Pediatrics, 2009; 156: 16-9

20. Mc Elrath T, Fichorova R, Allred E et al. Blood protein profiles of infants born before 28th weeks differ by pregnancy complication. Am J Obstet Gynecol, 2011; 204: 1-12

21. Blanco-quiros A, Arranz E, Solis G. Corb blood interleukin-10 levels are increased in preterm newborn. Eur J Pediatr, 2000; 159: 420-3

22. Dembinski J, Martini R, Behrendt D et al. Modulation of pro-and anti-inflammatory cytokine production in very preterm infants. Cytokine, 2003; 21(4): 200-6

23. Schultz C, Temming P, Bucsky P et al. Immature anti inflammatory response in neonates. Clin Exp Immunol, 2004; 135: 130-6

24. Natarajan G, Shankaran S, Mc Donald S et al. Circulating_Chemokine and MMP 9 as Markers of Oxidative Injury in Extremely Low Birth Weight Infants. Ped Res, 2010; 77-82

CHAPTER 7

Cytokines Patterns in Newborn Infants with Late Onset Sepsis

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Abstract

Background. Cytokines might be helpful to diagnose late onset sepsis (LOS) in newborn infants. Most studies compared infants with a culture proven and suspected sepsis vs controls. However, a clinically useful test should differentiate between a proven and clinical sepsis.

Objectives.To evaluate if cytokines can differentiate between proven and severe/mild clinical LOS.

Methods. Newborn infants with suspected LOS were enrolled in a prospective study between October-2007 and October-2009 in NICU of Harapan Kita Women and Children's Hospital-Jakarta,Indonesia. Twenty-five cytokines were measured. Infants more than 72 hours of age with proven sepsis(PS)(n=18), severe-clinical-sepsis(SCS)(n=6), mild-clinical-sepsis(MCS)(n=23) and controls(n=25) were included. Cytokines were measured at time when infection suspected and 4, 12, 24, 48 hours thereafter by Invitrogen-immunoassays-Luminex^{TM}100 systems.

Results. There was no significant differences in clinical characteristics between group PS and SCS, MCS had less clinical signs. Compared to controls, IL-1b, IL-1Ra, IL-2r, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-15, IL-17, TNFα, IFNa, MIP-1a, MIP-1b, IP-10, MIG, MCP1 were higher in group PS, IL-1ra, IL-2r, IL-8, IP-10, MIG were higher in group SCS, only IL-6 and IP-10 were higher in group MCS. IL-4, IL-5, IL-15, MIP-1a, MIP-1b were higher in group PS vs SCS. MIP-1a (at t=0,24 hrs) and IL-15 (at t=0,12,24 hrs) showed no overlaps in results between proven and both clinical-sepsis group and a good/high sensitivity and specificity.

Conclusions.MIP-1a and IL-15 might be good markers to detect a proven LOS. When these cytokines are not elevated in sick infants, other causes than an infection must be looked for.

Key words: neonates; Late onset sepsis; cytokines; chemokines. Abbreviations: PS,Proven-Sepsis; SCS,Severe-clinical-sepsis; MCS,mild-clinical-sepsis; LOS,late-onset sepsis; EOS,early- onset sepsis; IL,interleukin; AS,Apgar-Score; TNFα,Tumor necrosis factor-alpha; IFNɣ,Interferon gamma; CRP,C-reactive proteine; TH,T-helper cell; NK-cell,natural-killer cell; MCP1,monocyte-chemoattaractant proteine1; NEC,Necrotizing enterocolitis; MIP1a/b,Macrophage inflammatory proteine-1a/b.

Introduction

Infections in newborn infants are important contributors to morbidity and mortality in developed as well as developing countries[1]. In Indonesia, the neonatal mortality at present is 33,9 per 1000 live births, comparable to other developing countries[2,3,4]. Gram-negative infections are the most important causes of mortality[1,3,4,5]. Antibiotics are used very frequently in case of a suspected neonatal infection[4,5,6].

At the moment it is very difficult, if not impossible, to differentiate between a detoriation in the clinical condition of a newborn infant due to a bacterial infection or due to other causes. Clinical symptoms and laboratory investigations such as white-cell count and I/T-ratio are not conclusive. CRP has been shown to have an inadequate sensitivity and specificity and elevated levels may not be detected in the first 24 hrs after the first signs of a possible infection[8,9,10]. A recent study showed that Procalcitonin/PCT had a sensitivity of 60-88% and a specificity of 54-80%, depending on the cut-off level.[11] Since the results of a blood culture are only available 48 to 72hrs after the onset of symptoms, it is often impossible to wait antibiotics treatment until the blood culture results are available[9]. This implies that antibiotics are given to all suspected of infected infants. A retrospective survey of 662 newborn infants (GA=24-42wks) admitted to the NICU of Beatrix Children's Hospital in Groningen,Netherlands in 2004-2005 found that 42 newborn infants received antibiotics per case of confirmed early onset sepsis/EOS. The reported incidence of EOS was around 1.5%, comparable to other centres in developed countries.[12] In developing countries antibiotics are not only used to treat but also to prevent infections[4,7]. However, extensive use of antibiotics will induce resistance[7]. Methods to reduce the use of antibiotics are therefore important.

Incorporation of cytokines in the diagnostic work-up in infants with the suspicion of LOS might reduce the use of antibiotics. Different studies have shown that IL-6,IL-8,IL-10,TNFα and IP-10 are increased in infants with late-onset sepsis compared to controls.[9,13-22] Despite these reports, these interleukines are still not used in clinical practice to diagnose a neonatal infection. There might be a number of reasons why the results of these studies have not been implemented in clinical practice. Most studies were not able to differentiate between infants with a proven sepsis and infants

with a clinical sepsis without a positive blood culture. In many of the studies infants with suspected sepsis, regardless whether the result of the blood culture was positive or not, were compared to infants without signs of an infection. A useful test should, however, differentiate between infants with clinical signs of an infection and a positive blood culture versus those with the same signs but with a negative culture. This is important not only to assess the need for antibiotics, but also to know if other causes than an infection should be considered.

Studies of cytokines other than IL-6,IL-8,IL-10,TNFα and IP-10 have not shown that they were able to distinguish patients with proven sepsis from those with negative blood culture. Damman etal[23] were the first to analyze 16 cytokines in the first week of life in ELBW infants. They found higher levels of IL-1,IL-4,IL-6,IL-8,IL-10,IL-11,TNFα,MIP-1a and MIP1-b during the first 2-3 days of life compared to day-five. Unfortunately no clinical data of the infants regarding the presence or absence of an infection were available.

Finally it is known that IL-6,IL-8 and TNFα decrease rapidly after the onset of neonatal sepsis, possibly due to the administration of antibiotics. Most studies evaluated levels of cytokines at the moment of infection and 24 hrs later.[24-26] Thereby the moment of the highest value of cytokines/chemokines might have been missed.

The aim of the present study was to determine levels of 25 cytokines/chemokines in blood from newborn infants taken at the onset of suspected infection as well as 4,12,24,48 hrs later and comparing levels of these cytokines between infants with or without a positive blood culture. Secondly, levels were compared to results obtained in infants without an infection. The diagnostic capacity of these tests to distinguish these groups of infants was assessed. Finally, we aimed to detect the most optimal time point for the measurement of these cytokines/chemokines.

Patients and Methods

This is a prospective study on cytokine/chemokine levels in newborn infants conducted at the NICU of Harapan-Kita Women and Children Hospital,

Jakarta,Indonesia. All infants admitted to our unit from October-2007 until October-2009 with the suspicion of neonatal sepsis, both early and late onset sepsis, and without a major congenital abnormality were included. Infants were suspected of infection when they showed at least two clinical signs, i.e: increasing frequency of apnoea accompanied by either desaturation or persistent bradycardia (HR<60/min) or tachycardia (HR>160/min), respiratory dysfunction with requirement of respiratory support with need more oxygen, poor capillary perfusion or hypotension with requirement of circulatory support, hyperirritability/lethargy, seizures, temperature instability (<36,6˚C or >37,2˚C on two occasions within 24 hours), feeding intolerance, abdominal signs of Necrotizing-Enterocolitis(NEC), hyperglycemia(>10mmol/L) and metabolic acidosis(BE<-10mmol/L). At the moment of a suspected infection a septic screening (complete blood count, C-reactive proteine and blood culture) was done. The blood culture was analyzed by the BACTEC method then broad spectrum antibiotics were given. Antibiotics were discontinued when the blood culture was negative at 72 hours in clinically stable infants. When infants showed a clinical deterioration, the second line antibiotic was prescribed after taking a new blood culture. Other cultures were done only on indication. The study was approved by the Ethical Research Commitee of Harapan Kita Women and Children Hospital, Jakarta, Indonesia and reported to the Ethical Research Committee of the University Medical Centre Groningen, The Netherlands.

For the present study, all infants who were >72 hours of age and showed clinical signs of a sepsis were selected. First the infants with a positive culture were identified and included in group Proven Sepsis(PS). Thereafter, infants with signs of an infection but a negative culture were divided by two investigators independently into two groups. Infants with at least three clinical signs were included in a group Severe Clinical Sepsis(SCS) and infants with two clinical signs were included in a group Mild Clinical Sepsis(MCS). Infants were included as control when they were clinically stable, >72 hrs of age, had no signs compatible with an infection except mild respiratory problems for which they had received CPAP in the first three days after birth and had a negative blood culture. In control infants, samples were taken daily from day 3 to 7 after birth. As we did not

observe a trend in any of the cytokines during this period, we used the median of these daily measurements as control values.

Cytokine measurement

When patients presented with a suspected infection as well as 4,12,24 and 48 hours later, 0,3 ml blood was taken for cytokine levels together with blood taking for routine clinical purposes. The blood sample was centrifuged; 50 uL of serum was taken and stored at minus 80˚C within 15-30min after blood collection. Frozen serum was shipped on dry ice to the laboratory of University Medical Center Groningen,Netherlands where they were analyzed. Sera were analyzed using Invitrogen's Multiplex Bead Immunoassay. In a 96 well plate samples were prepared by adding beads of defined spectral properties which were conjugated to protein-specific capture antibodies, incubation buffer to bind cytokines to the protein-specific capture antibodies and biotinylated detector antibodies. Finally, streptavidin conjugated to the light-sensitive fluorescent protein R-Phycoerythrin was added and cytokine concentrations were analyzed with the Luminex detection system(Luminex Corp.,Austin,Texas) using the programme StarStation2.3. By monitoring the spectral properties of the beads and the amount of associated R-Phycoerythrin(RPE) fluorescence, the concentration of proteins was determined. The following interleukines were measured: IL-1B, IL-1Ra, IL-2, IL-2r, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, TNFα, IFNa, IFNg, IP-10, MIP-1a, MIP-1b, Eotaxin, Rantes, GM-CSF, MIG, MCP-1.

Statistical analysis

We compared serum cytokine levels at all time points (0,4,12,24,48 hours) between all groups. Non-parametric test(Mann-Whitney U) from a computerised database (SPSS-11 for windows,SPSS Inc,2001) was used to compare cytokine/chemokine values between PS vs. control, SCS vs. control, MCS vs. control, PS vs.SCS, PS vs.MCS and SCS vs. MCS. Because of multiple comparisons, significance was set at $p<0.01$, 95% of confidence intervals were calculated. Based on the receiver operating characteristic (ROC), sensitivity, specificity and positive and negative predictive values were calculated.

Results

During the two-years period of our prospective study, we collected samples from 201 neonates with suspected early or late onset infection. Fifty-six patients were excluded because of fatal congenital anomalies, parents refusal for further admission to the NICU, insufficient amounts of blood obtained or death during the study period due to causes other than infection.

For the present study we selected forty-seven neonates of more than 72 hours of age who showed minimally two clinical signs compatible with an infection. From these patients, based on the criteria given in the method section, eighteen were included in group PS, six in group SCS and twenty-three in group MCS. In group PS, 2 infants had gram-positive bacteria, 10 gram-negative, 2 candida, 2 infants with a meningitis and 2 infants NEC stage 3. Thirty-four of the infants were included into the control group.

Clinical characteristics of the four groups are presented in table1. There were no significant differences in gestational age, birth weight, gender and AS of less than 5 at 5 minutes among the groups although infants in group MCS tended to have a higher gestational age and birth weight. Clinical signs, routine laboratory investigations, duration of antibiotics and duration of admission in the NICU were not different between groups PS and SCS, except temperature instability, which was more frequent in the PS group. The CRP levels taken at time when infection suspected were not significantly different between groups PS and SCS, confirming that both groups were clinically comparable at the onset of the symptoms. According to the study design, infants in group MCS showed fewer symptoms compared to infants in group PS and SCS. CRP in group MCS was lower compared to PS [18 pg/ml(14-34) vs 61pg/ml(24-125)]. In group PS three infants died after the sampling period but before day 7.

Cytokines/chemokines were detectable in all patients at almost all time points. RANTES,IL-2,IL-12,IL-13,Eotaxin and GM-CSF did not show a difference in levels between groups. The levels of other cytokines/chemokines are shown in Table 2.

Compared to the control group, higher values in group PS were found for IL-1B,IL-1Ra,IL-2r,IL-6,IL-7,IL-8,IL-10,IL-15,MIP-1a,MIP-1b,IP-10,MCP-1 at all time points, IL-17 at 0,4 hrs, IL-4 at 0,4,12 hrs, IL-5 at 12,24,48 hrs, TNFα at 4,12 hrs, IFNa at 4,12,24 hrs, MIG at 0,4,12,24 hrs and IFNg at 12hrs. When comparing SCS to controls, higher levels were found in group SCS of IL-8 at all time points, MIP-1a,IL-2r,IL-6 at 0,24 hrs, IFNa at 4 hrs, IL-5 at 48 hr, IP-10 and MIG at 0,4,12 hrs. Group MCS showed higher levels compared to controls of IP-10 at all time points except 4 hrs, IL-6 at 0,4,12 hrs, IL-2r and IL-7 at 4 hrs and MIG at 0 hr.

Compared with group SCS, higher values in group PS were found for IL-4 at 0 hrs, IL-5 at 24-48 hrs, IL-15 at 0,12-24 hrs, MIP-1a at 0,24-48 hrs and MIP-1b at 12 hrs. When comparing between group PS and MCS, higher values in group PS for IL-6 and MIP-1a at all time points, IL-Ra and IL-15 at all time points except 4 hrs, IL-7 and IL-17 at 24 hrs, IL-8 at 12 hrs, TNFα at 4,24-48 hrs, MIP1-b at 0,4,48 hrs and MCP-1 at 4 hrs were found. We found no significant diferences in cytokine levels between SCS and MCS. Comparing PS to the other groups, there was no overlap in levels of IL-15 at 0,12,24 hrs and MIP-1a at 0,24 hrs as shown in figure 2.

Table1. Characteristics of study groups

Characteristics	Control (n=34)	MCS (n=13)	SCS (n=6)	PS (n = 18)
GA (wks),median (25-75 th)	34 (32-34)	36 (30-39)	33 (29-36)	32 (25-34)
BW (gram), mean (range)	1958 (1480-2035)	2300 (1328-3352)	1800 (1057-2560)	1700 (1134-2350)
C-Section	20	13@	5	10
Gender: Male	11	10	3	8
AS less than 5 at 5 min	1	1	2	5
Day first symptoms (days)	0	4	4	7
Temperature Instability	0	6@	2*	15*
ABS	0	2	5	8
Increasing of need oxygen	0	3	5	9
Mechanical ventilation	0	3@	5	12
Nasal CPAP	21	8@	1	1
Clinical seizure	0	0@	1	6
Cardioresp. insufficiency	0	3	1	10
GI tract problems	0	4	0	5
Leucocyte < 5000 or > 30.000/mm3	0	3	0	8
Thrombocyte < 150.000 or > 600.000/mm3	4	1	1	10
CRP (mg/dl)	4 (0-4)	18 (14-34)@	23(8-38)	61(24-125)
Day antibiotics ,median	4 (4-5)	7 (6-12)	12 (7-14)	16 (10-20)
Mortality within 7 days	0	0	0	3
Day admission at NICU,median	8 (6-16)	10 (7-15)	27 (19-47)	15 (12-28)

Data shown as median, with a range: min-max; ABS = Apnoea,bradycardia,desaturation
used Fisher Exact test : * :p < 0.05, between PS and SCS ; @:p <0.05, between PS and MCS .
PS,proven sepsis ; SCS, severe clinical sepsis; MCS, mild clinical sepsis

Table 2. Serum concentrations of 19 cytokines at t=0,4,12,24,48 hrs of study groups. Data shown as median(25th-75th percentiles)

Cytokines	Time	Control (n=34)	MCS (n=13)	SCS (n=6)	PS (n=18)
IL-1B	0		10 (1-45)	21 (5 – 97)	47 (34 – 66)%
	4	4 (4 – 14)	30 (1-64)	21 (1 – 93)	47 (37 – 59)%
	12		24 (1-58)	26 (1-125)	50 (42-142)%
	24		1 (1-52)	18 (1-131)	47 (45-180)%
	48		1 (1-64)	22 (8-53)	42 (25-108)%
IL-1Ra	0	441 (235 - 587)	503 (263-1504)	3420 (1049 - 5206)	19790 (2991 - 48535)%@
	4		575 (338-1100)	473 (172 - 2051)	20195 (1156 - 41032)%@
	12		712 (295-2198)	473 (274 - 1535)	9418 (1354 - 37277)%
	24		467 (293-724)	458 (228-1413)	2659 (1001-18322)%@
	48		481 (208-852)	360 (181-2242)	5631 (389-18759)%
IL-2r	0		1122 (376-1771)	1193 (982 – 1371)#	2234 (1113 – 4250)%
	4	610 (521-716)	1540 (587-1843)&	1039 (937 – 1387)	2551 (1058 – 4015)%
	12		1254 (510-1833)	1222 (988 – 1272)	2430 (1039 – 4273)%
	24		1397 (402-1749)	1139 (791-1536)#	1996 (935-4249)%
	48		1307 (473-1697)	1206 (788-1304)	1993 (1171-4304)%
IL-4	0	3 (1-6)	1 (1-35)	7 (3 - 9)	26 (18- 81)%*
	4		7 (1-63)	7 (4 - 7)	39 (10 - 113)%
	12		4 (1-34)	7 (4 - 7)	31 (7 - 78)%
	24		1 (1-23)	5 (1-10)	20 (2-94)
	48		1 (1-54)	7 (3-12)	12 (3-90)
IL-5	0	6 (6 – 4)	1 (1-33)	2 (1 – 7)	35 (7 – 37)
	4		3 (1-32)	2 (1 – 3)	32 (4 – 37)
	12		3 (1-33)	2 (2 – 8)	31 (10 – 37)%
	24		1 (1-32)	1 (1-5)	37 (30-37)%*
	48		2 (1-33)	1 (1-4)#	35 (15-37)%*

Cytokines	time	Control (n=34)	MCS (n=13)	SCS (n=6)	PS (n=18)
IL-6	0	4 (2-10)	16 (10-30)&	26 (13 - 112)#	223 (82 - 2294)%@
	4		15 (7-26)&	46 (12 - 86)	149 (72 - 869)%@
	12		16 (7-27)&	42 (7 - 198)	148 (94 - 381)%@
	24		11 (5-20)	23 (15-212)#	85 (53-384)%@
	48		10 (5-18)	53 (6-167)	117 (36-619)%@
IL-7	0	1 (1-10)	1 (1-20)	11 (2-20)	25 (10-56)%
	4		10 (1-61)&	11 (11-33)	39 (2-88)%
	12		6 (1-29)	1 (1-2)	45 (10-54)%
	24		1 (1-7)	2 (12-28)	23 (10-47)%@
	48		2 (1-61)	1 (1-2)	11 (1-52)%
IL-8	0	39 (18 - 78)	32 (23-145)	304 (100 - 1012)#	187 (91 - 751)%
	4		64 (35-140)	251 (141 - 1076)#	187 (65 - 797)%
	12		47 (30-93)	286 (204 - 767)#	202 (85 - 875)%@
	24		50 (24-120)	282 (59-1609)#	151 (72-635)%
	48		57 (23-74)	173 (95-1836)#	184 (45-647)%
IL-10	0	4 (2 – 21)	14 (11-27)	26 (17 – 446)	207 (34 – 849)%
	4		16 (13-29)	23 (13 – 562)	74 (19 -496)%
	12		16 (13-28)	26 (14 – 61)	41 (15 – 75)%
	24		15 (12-23)	20 (9-30)	24 (11-90)%
	48		15 (12-21)	19 (14-599)	57 (12-154)%
IL-15	0	36 (23 - 47)	17 (1-49)	29 (18 - 38)	144 (72 - 368)%*@
	4		10 (1-42)	19 (7 - 29)	152 (41 - 336)%
	12		18 (1-51)	22 (13 - 25)#	122 (67 - 223)%*@
	24		10 (1-40)	11 (1-50)	89 (55-549)%*@
	48		10 (1-46)	19 (10-45)	122 (62-538)%@

Cytokines	Time	Control (n=34)	MCS (n=13)	SCS (n=6)	PS (n=18)
IL-17	0	21 (6 – 32)	3 (1-5)	21 (10 – 30)	73 (36 – 127)%
	4		2 (1-9)	16 (14 – 23)	46 (27 -152)%
	12		4 (1-38)	23 (18 – 35)	36 (27 – 139)
	24		1 (2-5)	18 (5-46)	30 (19-156)@
	48		1 (1-5)	33 (1-71)	27 (2-115)
TNFα	0	4 (1-5)	1 (1-5)	2 (1 - 2)	4 (4 - 13)
	4		2 (1-4)	3 (1 - 3)	7 (4 - 28)%@
	12		2 (1-6)	3 (1 - 3)	8 (4 - 22)%
	24		2 (1-3)	1 (1-2)	5 (4-32)@
	48		2 (1-6)	3 (1-5)	13 (4-22)%@
IFNa	0	40 (30-51)	38 (33-73)	106 (69-125)	56 (53-104)
	4		49 (26-67)	98 (66-112)#	66 (56-81)%
	12		56 (44-62)	98 (91-112)	77 (56-107)%
	24		51 (40-74)	91 (55-109)	65 (56-107)%
	48		46 (33-63)	91 (66-109)	61 (56-376)
IFNɣ	0	7 (7 – 8)	2 (1-131)	5 (4 – 9)	18 (3 – 146)
	4		6 (2-282)	4 (2 – 7)	46 (2 – 88)
	12		4 (2-165)	5 (4 – 8)	24 (12–58)%
	24		2 (1-134)	9 (3-11)	24 (4-92)
	48		2 (1-141)	7 (2-21)	18 (8-73)
MIP1a	0	28 (24 – 33)	37 (22-98)	52 (36 – 65)#	217 (115 – 296)%*@
	4		53 (25-112)	42 (39 – 53)	179 (87 – 262)%@
	12		38 (26-123)	40 (31 – 57)	172 (96 – 257)%@
	24		30 (21-92)	43 (31-56)#	143 (101-316)%*@
	48		29 (23-122)	48 (38-63)	203 (101-362)%*@

Cytokines	Time	Control (n=34)	MCS (n=13)	SCS (n=6)	PS (n=18)
MIP1b	0	60 (38 – 94)	70 (39-115)	91 (55 – 297)	339 (103 – 1452)%@
	4		120 (30-151)	105 (54 – 156)	310 (220 – 732)%@
	12		178 (37-321)	99 (54 – 124)	310 (218 – 517)%*
	24		106 (65-173)	62 (27-250)	250 (116-996)%
	48		53 (27-167)	79 (45-162)	230 (160-1841)%@
IP-10	0	43 (18 - 93)	189 (50-595)&	376 (296 - 518)#	107 (58 - 410)%
	4		126 (29-526)	334 (301 - 448)#	262 (80 - 802)%
	12		210(34-532)&	191 (157 - 274)#	184 (73 – 1256)%
	24		128 (43-523)&	232 (77-556)	159 (40-1272)%
	48		170 (43-545)&	179 (134-1530)	279 (53-1347)%
MIG	0	23 (4 - 57)	89 (16-163)&	179 (87 - 213)#	201 (59 - 442)%
	4		89 (16-193)	206 (149 - 545)#	236 (67 - 605)%
	12		61 (43-129)	179 (164 - 179)#	194 (76 - 345)%
	24		75 (18-129)	134 (107-1918)	81 (55-274)%
	48		75 (34-141)	81 (54-274)	277 (40-383)
MCP1	0	362 (195- 680)	297 (238-629)	868 (465 - 1569)	1461 (756 - 5460)%@
	4		674 (168-967)	563 (536 - 1717)	1017 (571 - 6007)%
	12		495 (220-814)	650 (609 - 765)	735 (623 - 1797)%
	24		613 (176-1010)	755 (156-2245)	786 (372-2377)%
	48		365(43-708)	771 (382-5183)	749 (462-2711)%

Data shown as median (25-75 percentiles);

MCS, Mild clinical sepsis; SCS, severe clinical sepsis; PS, proven sepsis

Between PS and SCS, * : $p < 0.01$ (CI 95%), Mann-Whitney U test

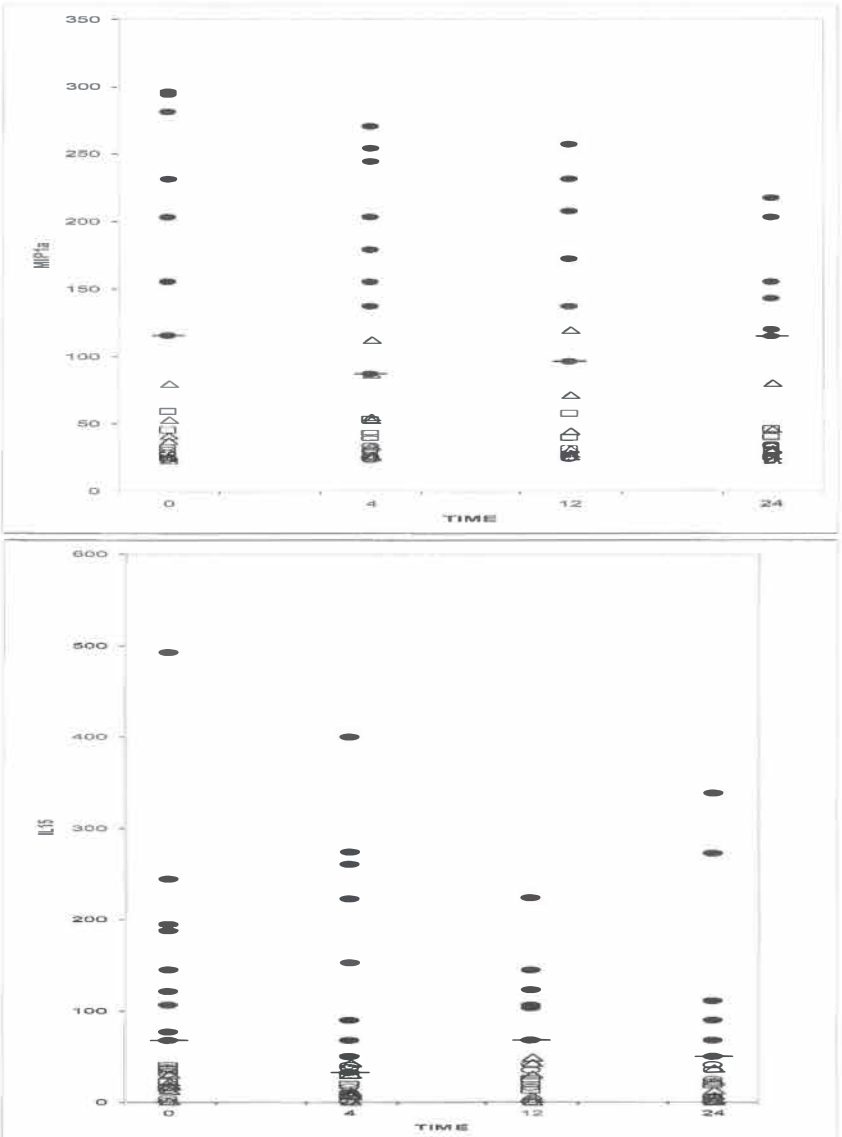
Between PS and MCS,@: $p<0.01$ (CI 95%), Mann-Whitney U test

Between PS and Control , % :$p<0.01$ (CI 95%), Mann-Whitney U test

Between SCS and Control, # :$p<0.01$ (CI 95%) , Mann-Whitney U test

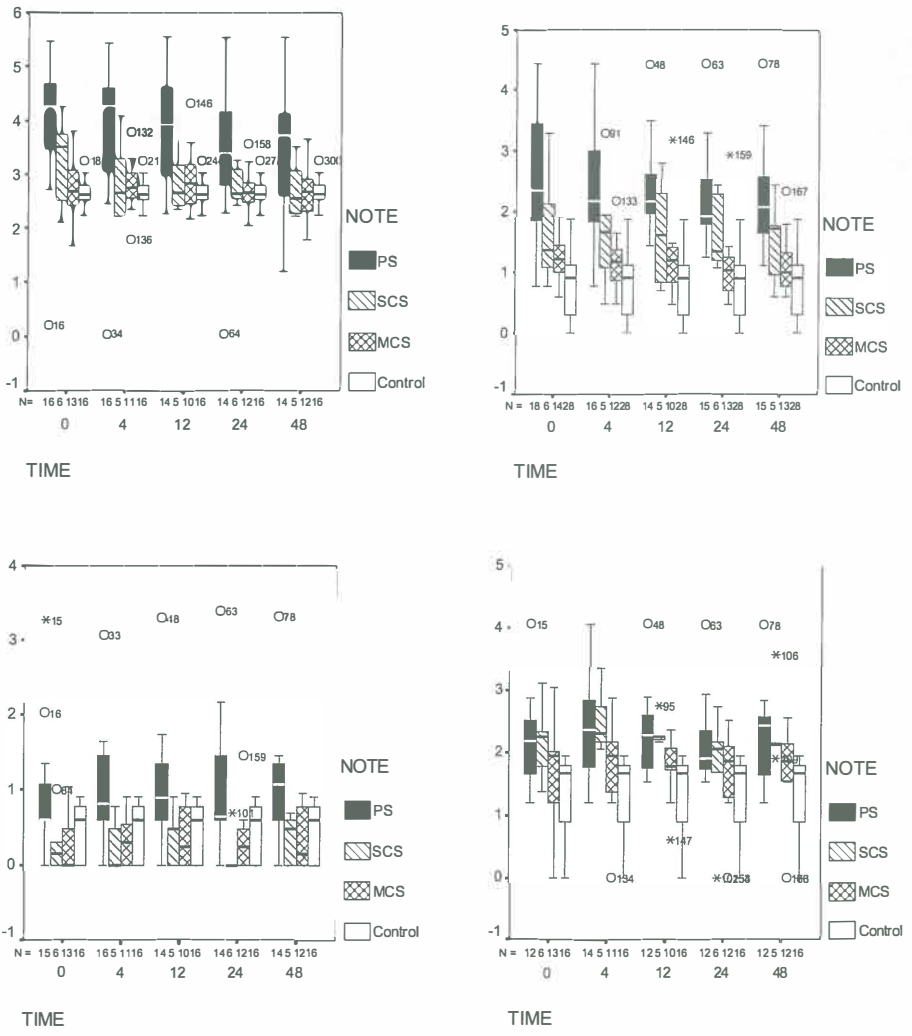
Between MCS and Control, & :$p<0.01$ (CI 95%),Mann-Whitney U test

Figure 1. Serum concentrations of MIP-1a and IL-15 of infants at 0,12,24,48 hours



● = Proven ; □ = SCS ; △ = MCS ; ○ = Control

Figure 2. The trends of levels IL-1Ra,IL-6,TNFα,MIG of study groups. Data shown as median(25-75 percentiles) on logarithmic scale.



Sensitivity, specificity, positive and negative predictive value calculated from the ROC curves to distinguish between proven and clinical sepsis are shown in table 3. IL-1Ra, IL-4, MIP-1a and IL-15 show good to high sensitivity and specificity at 0 hr, IL-4, IL-6, MIP-1a and IL-15 at 24 hr. As shown in table 2, almost all cytokines, in all three groups, did not show a

trend during the 48 hrs observation period, except IL-1Ra,IL-6,MIG,TNFα (figure 1). In group PS, IL-1Ra was higher at 0 and 4 hrs compared to later time points, IL-6 showed a decreasing trend over time but all these levels remained higher then control values. MIG showed a decreasing trend over the first 24 hr and TNFα started to increase only at 4 hrs. In group SCS, IL-1Ra was higher only at 0 hr compared to all later points and IL-6 did not show a trend over time. In group MCS, IL-6 was elevated during the first 12 hrs, thereafter the levels were not different from controls.

Table3. The sensitivity, specivicity, positive and negative predictive value (PPV and NPV) of IL-4,IL-5,IL-6,IL-15,MIP-1a and MIP-1b between proven sepsis (PS) and combined of both clinical-sepsis (SCS and MCS) group

Cytokines Cut off value (hrs)	Sensitivity	Specivicity	PPV	NPV	Area under ROC (95% CI)
IL-1Ra					
> 652(0)	94	52	62	91	85 (73-97)
> 567 (24)	86	67	67	86	79 (62-97)
IL-4					
>16 (0)	94	85	80	96	90 (80-100)
>24 (24)	100	87	50	100	93 (78-100)
IL-5					
>9 (0)	88	73	68	91	75 (64-93)
>33 (12)	100	92	95	100	96 (90-100)
IL-6					
> 18 (0)	**85**	**64**	**58**	**86**	**80 (66-94)**
>31 (24)	**99**	**82**	**50**	**100**	**88 (71-100)**
MIP-1a					
> 81 (0)	**87**	**85**	**79**	**92**	**91 (82-100)**
>69 (12)	**100**	**76**	**89**	**100**	**96 (90-100)**
> 96 (24)	**100**	**88**	**50**	**100**	**93 (79-100)**
>58 (48)	**91**	**72**	**71**	**92**	**87 (74-100)**
MIP-1b					
>85 (0)	91	60	58	89	80 (67-93)
>124 (12)	92	62	82	80	79 (64-95)
>145 (24)	100	74	33	100	87 (60-100)
IL-15					
>20 (0)	**90**	**55**	**56**	**88**	**86 (74-98)**
>63 (12)	**100**	**97**	**95**	**100**	**100 (100-100)**
>76 (24)	**100**	**91**	**50**	**100**	**96 (88-100)**
>48 (48)	**91**	**79**	**78**	**92**	**88 (73-100)**

Discussion

In this study we show that the levels of 19 out of the 25 tested interleukins are higher in infants with a proven late onset sepsis compared to non-infected patients. In infants with clinical symptoms of an infection but a negative culture, the increase in cytokines levels is related to the severity of illness. Infants with three or more symptoms showed elevated levels compared to controls in fifteen, compared to eight interleukins in infants with two symptoms. A significant difference in levels of IL-4,IL-5,IL-15,MIP1-and MIP1-b were found between infants with a proven and severe clinical sepsis. IL-15 and MIP-1a had no overlap between PS and other groups. Only IL-1Ra,IL-6,TNFα and MIG showed a trend over time.

A number of studies have compared levels of cytokines/chemokines between infants with a proven or clinical LOS versus non-infected infants.[16-18,20,22-26] Most studies measured levels of IL-6, IL-8,IL-10,TNFα and IP-10. De Bont etal[15] were one of the first to show elevated levels of IL-6,IL-1b and TNFα in infants with LOS. They compared infants with proven, mainly gram-positive infections, or the combination of proven and suspected cases to controls. Kuster etal[24] found significantly elevated levels of IL-1Ra and IL-6 in infants with a proven sepsis compared to controls. These levels became elevated one or more days before the onset of the infection. The group of infants with signs compatible with an infection but without a positive culture showed intermediate results for both cytokines, and no significant differences with either the proven sepsis or control group were observed. Ng etal[17] compared levels of IL-1b,IL-6,IL-8,IL-10,IL-12,IP-10,MIG,MIP-1 and TNFα between infants with a proven sepsis and controls. They observed significantly higher levels of IL-6,IL-8,IL-10,IP-10,TNFα,MIG and MIP-1 at the onset of disease. IL-6,IL-10,IP-10 and MIG were still elevated at the moment of the second blood sampling, 24 hrs after the first sample. In another study by Ng etal[18], higher concentrations of IL-2,IL-4,IL-6,IL-10,IFNg and TNFα were found at the onset of symptoms in infected infants than in those not infected. For most cytokines, however, there was a rather wide range in levels with a clear overlap between both groups. In a recent study, Hotoura etal[27] measured IL-6,IL-1b and TNFα in term infants and the moment of suspicion of an infection and two days later. Higher levels of all cytokines were found in

both proven and suspected infants with less increase in the suspected to the proven group. No data as to the severity of illness in the suspected group was shown.

Previous studies indicated that IL-6 might not only be increased in due to bacterial infections, but also to other causes of illness. Harris etal[28] found a five to tenfold higher IL-6 level in infants with sepsis and NEC as compared to sepsis alone or NEC with a negative blood culture. Dollner etal[16] showed higher IL-6 levels in infants with proven compared to clinical sepsis, the severity of illness however was lower in the clinical sepsis group. Buck etal[19] did not find a difference in IL-6 levels between infants with proven sepsis compared to infants with three or four clinical signs compatible with infections. These results are in agreement with a study in adults where IL-6 and TNFα were measured in patients with septic shock and trauma. IL-6 was elevated in both groups, although it was higher in the sepsis group. In contrast, TNFα was only elevated in the sepsis group.[29] Our results confirm these findings that IL-6 increases due to many factors, not only bacterial infections. Our data indicate that IL-6 can not distinguish between proven and suspected sepsis at the onset of symptoms, at 24 hr however IL-6 can be used to differentiate between proven and suspected infection.

If IL-8 and IL-10 can differentiate between infants with a positive culture and either sick infants without a positive culture or controls is not yet clear. Gonzales etal[30] found no differences in IL-8 levels at time 0 and 24 hours between infants with proven LOS compared to controls. Ng etal[18] found higher IL-8 levels in infants with proven LOS than controls. We found higher IL-8 levels in group PS and SCS, but levels in group MCS were not higher compared to controls. This indicates that an increase in IL-8 is more due to illness than bacterial infection. Romagnoli etal[14] showed that IL-10 was higher at presentation of clinical signs of an infection and 12 hours later in infants with proven sepsis vs. controls. No difference was observed between infants with proven and suspected sepsis. Ng etal[18] found higher IL-10 levels in preterm infants with infection vs controls, while levels in infants with clinical signs of infection and negative culture were not increased. In another study by the same group, IL-10 levels were higher in suspected infants vs. those not infected at 0 and 24 hr, but their levels were significantly lower compared to septic infants.[22] In our study, IL-10 was

elevated in both group PS and SCS compared to control, reached significant different only in group PS. Levels between group PS vs SCS/MCS were not different. We conclude that IL-10 is not a specific and sensitive marker for bacterial infection in newborn infants. If IP-10 can detect infants with proven sepsis was, so far, only studied by Ng etal[17] who found IP-10 to be good marker for LOS. In our study the IP-10 levels were elevated in all three groups compared to controls and without a difference between groups. It might well be that the elevation of IP-10 is not really due to infection. Ng etal[17] found that infants with sepsis complicated by intravascular coagulation (DIC) showed elevated levels, while levels in infants with sepsis without DIC were less increased.

TNFα, a proinflammatory cytokine, is secreted predominantly by monocytes in response to inflammatory stimuli. A study in adults showed that TNFα was increased in all patients with septic shock and not increased in severely ill patients with trauma.[29.] Girardin etal[31] showed that neonates can produce TNFα as well as adults, in respons to endotoxaemia. They showed higher levels of TNFα in neonates with bacterial sepsis than in neonates with only bacterial colonization. De Bont etal[15] showed that TNFα levels may be influenced by antibiotic treatment, the level was decreased significantly 8 hours after treatment. In our study, the levels of TNFα were markedly lower vs the level in Sharma etal (4vs.195pg/ml,respectively).[32] We do not know If these low levels in our study were caused by antibiotics prescribtion. The levels in our study were not higher in group PS compared to SCS. We can not confirm that TNFα is a good marker to detect severely ill newborn infants with proven LOS.

IL-4 and IL-5, both are anti-inflammatory cytokines. Ng etal[18] found a higher level of IL-4 at onset of infection in infants with proven infection compared to controls and no difference in levels of IL-5. Our results are in line with Ng study. We found higher levels of both cytokines in group PS compared to SCS, IL-4 at when infection suspected and IL-5 at 24-48 hrs later. IL-4 showed good sensitivity and specificity at t=0,24 hrs but with overlapping between PS and other clinical sepsis group. IL-5 showed poor diagnostic performances. Therefore IL-4 and IL-5 is not well suited to identify newborn infants with proven LOS.

MIP-1a and MIP-1b are proinflammatory cytokines, defined as B-chemokines, involved in the initiation and propagation of the inflammatory response. MIP-1a and MIP-1b activate IL-1,IL-6 and TNFα production.[33,34] Fotopoulos etal[35] showed higher levels of MIP-1a in infants with nosocomial infection compared to infants with perinatal asphyxia. In our study, MIP-1 had higher levels at all time points in group PS compared to both clinical sepsis groups, with no overlap at 0 and 24 hrs. MIP-1b only differentiated between PS and SCS at 12 hrs with an overlap between PS and MCS. MIP-1a taken at 0 and 24 hrs can be a good infection marker with the following reasons. First, the levels in proven sepsis are not only elevated at the time when infection suspected but still elevated at 24 hours with no overlap with both clinical groups. Therefore, levels of MIP1a in proven sepsis had good sensitivity and specivicity. Finally, MIP-1a is the first cytokine to increase in response to bacterial infections and not dependent on other cytokines.

IL-15 is considered to be able to stimulate natural killer cells and the killing activity. Natural killer cells are part of innate immune system and play an important role in infections and auto immune diseases.[36,37] IL-15 showed higher levels in group PS compared to SCS and MCS without overlap at 0,12,24 hrs. We can not explain why levels in group SCS were lower compared to control. The increased level of IL-15 in the proven septic infants might indicate a good performance of activity of NK-cell, which could be assumed as a response to the infection. A second conclusion is that neonates can produce IL-15 as well as adults. As far as we know, no other study analyzed this cytokine in LOS infants. To confirm our findings further studies regarding the use of IL-15 as indicator of LOS are needed.

A number of studies evaluated serially cytokines, at the onset of infection and 24 hrs later. Kuster etal[23] showed that IL-6 was increased at the onset of sepsis and returned to control levels at 24 hrs. Ng etal showed a lower level of IP-10,MIG,IL-6 and IL-10 in culture proven infants at 24 compared to 0 hr, although levels at that moment were still higher than controls. We also found that IL-6 and IL-10 was lower at 24 hr in group PS compared to t=0 hr. Only in group MCS IL-6 decreased during the first 24 hr to be not different anymore from controls at 24 hr. Our results indicate that the diagnostic performance of cytokines will not improve with more sampling in

the first 24 hr after onset of symptoms. Sampling at 24 hrs can be used to confirm the results of t=0. When IL-6 is not higher than control values at 24 hrs in not severely-sick-infants, it can be used as indicator of a negative culture, together with the absence of an increase in MIP-1a.

Our study does have limitations. We conducted our studies in a developing country where the use of antibiotics is high, therefore gram negative bacteria were the main cause of LOS. Secondly we did not have a high number of infants in the severe clinical sepsis group. However, the patterns and differences of cytokines between groups were in line with previous studies. We suggest that our results therefore must be considered as a starting point for further studies.

In conclusions, In this study we show that five of twenty-five tested cytokines are higher in newborn infants with a culture proven LOS compared severely-ill-culture-negative infants. Ten cytokines were higher in culture proven LOS compared to mildly-ill-culture-negative infants. Due to overlapping values between groups, most cytokines are not sensitive enough to differentiate between ill-infants with and without a positive culture. MIP-1a and IL-15 measured at the onset of symptoms and 24 hrs later are able to detect sick infants with a bacterial infection. In sick infants without an increase in these cytokines other causes for the detoriation must be considered. More frequent determination of cytokines in the first 24 hrs after the onset of symptoms do not improve the predictive value.

References

1. Stoll BJ,Hansen N,Fanaroff AA etal. Late onset sepsis in very low birth weight neonates:The experience of the NICHD-Neonatal Research Network.Pediatrics,2002;110(2):285-91

2. Kementrian Negara Pembangunan Nasional Indonesia/Bappenas.Child Mortality.In: Indonesia Millenium Developmental Goal Reports.Jakarta, Indonesia,2007,pp.49-54

3. Lusyati S,v.d Broek,Sauer PJ. Neonatal sepsis in a NICU of Indonesia.J Hosp Infect.2009 Apr;71(4):383-5.

4. Zaidi AK,Huskins WC,Thaver D etal.Hospital-acquired neonatal infections in developing countries.Lancet,2005;365:1175-88

5. Turkish Neonatal Society-Nosocomial Infections Study Group.Nosocomial infections in neonatal units in Turkey:Epidemiology, problems, unit policies and opinions of healthcare workers.Turk J Pediatr,2010;52(1):50-7

6. Vergnano S,Menson E,Kennea N etal. Neonatal infections in England:The Neonatal Infection surveillance network.Arch Dis Child Fetal Neonatal Ed.,2011;96(1):F9-F14

7. Viswanathan R,Singh AK,Mukherjee S etal.Aetiology and Antimicrobial Resistance of Neonatal Sepsis at a Tertiary Care Centre in Eastern India:A 3-Year Study.Indian J Pediatr.,2010;17

8. Fowlie PW,Schmidt B.Diagnostic test for bacterial infection from birth to 90 days–a systematic review. Arch Dis Child Fetal and Neonatal Ed,1998;78(2):F92-8

9. Malik A,Hui CP,Pennie R. Beyond the Complete Blood Cell Count and C-Reactive Protein. A Systematic Review of Modern Diagnostic Tests for Neonatal Sepsis.Arch Pediatr Adolesc Med.2003;157:511-516

10. Garland and Bowmann.Reappraisal of C-reactive protein as a screening tool for neonatal sepsis.Pathology(2003)35,pp.240–243.

11. Auriti C,Fiscarelli E,Ronchetti MP etal.Procalcitonin in detecting neonatal nosocomial sepsis. Arch Dis Child Fetal Neonatal Ed.2011(15)

12. Stoll BJ,Hansen N,Fanaroff AA etal.Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants.N Engl J Med,2002;347(4):240-7

13. Blackwell TS and Christman JW.Sepsis and Cytokines: Current status.British J Anesthesia, 1996;77:110-17

14. Romagnoli C,Frezza S,Cingolani A etal.Plasma Levels of Interleukin-6 and Interleukin-10 in Preterm Neonates Evaluated for Sepsis.Eur J Pediatr,2001;160:345-50

15. De Bont, Martens A, van Raan S etal.TNFa,IL-1b and IL-6 Plasma Levels in Neonatal Sepsis.Pediatric Res,1992;380-3

16. Dollner H, Vatten L, Austgullen R. Early Diagnostic Markers for Neonatal Sepsis: Comparing C-reactive proteine,IL-6, soluble tumor necrosis factor receptors and soluble adhesion molecules.J Clinical Epidemiology,2001:1251-7

17. Ng PC, Li K, Chui KM etal.IP-10 is an Early Diagnostic Marker for Identification of Late Onset Bacterial Infection in Preterm Infants.Pediatric Res,2007;61:93-8

18. Ng PC,Li K,Wong RP etal.Proinflammatory and anti inflammatory cytokine responses in preterm infants with systemic infections. Arch Dis Child Fetal Neonatal Ed, 2003; 88: F209-13

19. Buck C,Bundschu J,Bartmann P etal.Interleukin 6:a Sensitive Parameter for the Early Diagnosis of Neonatal Bacteria Infection.Pediatrics,1994;93:54-8

20. Panero A,Pacifico L,Rossi N etal.Interleukin-6 in neonates with early and late onset infection.Pediatr Infect Dis J,1997;16(4):370-5

21. Groll AH,Meiser A,Weise M etal.Interleukin-6 as early mediator in neonatal sepsis.Pediatr Infect Dis J,1992;11(6): 496-8

22. Ng PC,Chui KM,Fok TF etal. Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecules, and C-reactive protein in preterm very low birthweight infant.Archives of Dis Childhood,1997;77:221-7

23. Damman O,Leviton A.Matenal Intrauterine Infection, Cytokine and Brain damage in the preterm newborn.Pediatr Res,1997;42(1):1-8.

24. Kuster H,Weiss M,Willeitner AE etal.Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestations.Lancet,1998; 352(9136):1271-7

25. Magudumana MO, Ballot DE, Cooper PA etal.Serial interleukin-6 measurements in the early diagnosis of neonatal sepsis.J Trop Pediatr,2000;46(5):267-71

26. Maciolek VM,Thijsen SF,Hemels M etal.Inflammatory mediators for the Diagnosis and Treatment of Sepsis in Early Infancy.Pediatric Res,2006;59:457-61

27. Hotoura E,Giapros V,Kostoula A.Tracking Changes of Lymphocyte Subsets and Pre-inflammatory Mediators in Full-term Neonates with Suspected or Documented Infection. Scandinavian Journal of Immunology,2011;73:250–255

28. Harris MC,Carl T,Gallagher P etal.Cytokine elaboration in Critically Infants with Bacterial Sepsis, NEC, or Sepsis Syndrome: Correlation with Clinical Parameters of Inflammation and Mortality.J Pediatr,2005;147:462-8

29. Martin C,Boisson C,Haccoun M.Patterns of cytokine evolution (tumor necrosis factor alpha and interleukin -6) after septic shock, haemorrhagic shock and severe trauma.Crit Care Med, 1997;25:1813-9

30. Gonzales BE,Mercado CK,Johnson L.Early markers of late onset sepsis in premature neonates: clinical, hematological and cytokine profile.J Perinat.Med.,2003;31:60-8

31. Girardin EP, Berner ME, Grau GE etal.Serum tumour necrosis factor in newborns at risk for infections.Eur J Pediatr,1990;149(9):645-7.

32. Sharma R,Tepas JJ,Hudak ML.Neonatal gut barrier and multiple organ failure:role of endotoxin and proinflamatory cytokines in sepsis nd necreotizing enterocolitis.J Pediatric Surgery,2006:454-61

33. Curfs JH,Meis JF and Korstanje H.A primer on cytokines: sources, receptors, effect and inducers.Clin Microbiology Review,1997:742-80

34. Luster AD. Chemokines–chemotactic cytokines that mediate inflammation. The New England Journal of Medicine,1998;338:436-445

35. Fotopoulos S,Mouchtouri A,Xanthou G etal. Inflammatory chemokine expression in the peripheral blood of neonates with perinatal asphyxia and perinatal or nosocomial infection. Acta Pediatr,2005;94(6):800-6

36. Fehniger TA,Caligiuri MA: Interleukin 15: biology and relevance to human disease. Blood,2001,97:14-32.

37. Casimir,Sylvie Ferrari-Lacraz,Jendly S.The proinflammatory cytokines IL-2,IL-15,IL-21 modulate the repertoire of mature human natural killer cell receptors. Arthritis Reaserch and therapy,2007;9(6):1-15

CHAPTER 8

Serial measurements of IL-6 and IL-8 in newborn infants with proven and suspected early onset sepsis.

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Abstract

Background: Neonates are frequently treated with antibiotics during their first days of life because of suspected early onset sepsis (EOS). Restrictive use of antibiotics is necessary given because of the low rate of blood culture proven EOS and possibility of antibiotic resistance. Cytokines are suggested to be able to detect neonatal sepsis, but data on serial measurements in the first hours of life are scarce.

Objective: To determine the usefulness of serial measurements of IL-6 and IL-8 in the first 48 hours of life to exclude a proven EOS.

Design/Methods: Newborn infants with suspected EOS were enrolled in a prospective study between July-2007 and December-2009 in two tertiary and two general hospitals. IL-6 and IL-8 concentrations were compared between infants with a bloodculture-proven EOS and control infants with maximal one sign of infection, but a negative bloodculture. Infants were matched for gestational age, perinatal risk factors and route of delivery. IL-6 and IL-8 concentrations were measured at 4 times periods: 0 – 4 hrs , 4 – 18 hrs, 18 - 30 hrs, and 42 - 54 hrs postnatally using a multiplex bead array assay at a Luminex 100 system (Luminex Corp., Austin, TX, USA).

Results: Six out of 510 included neonates suffered EOS ; *Group B streptococci* (n=5) and *Escherichia coli* (n=1) were cultured micro-organisms. 12 Neonates were selected for the control group. Median (range) gestational age was 36 (31-40) weeks. IL-6 concentrations were significantly higher in the EOS group compared to control infants at all time periods ($p < 0.001$). IL-8 showed an earlier decline and was higher at the first three time periods in neonates with EOS ($p < 0.01$).

Conclusions: Elevated levels of Il-6 were seen in infants with a proven sepsis till 60 hours, in infants with a clinical sepsis levels were higher up to 12 hours after start of symptoms. A pattern of low IL-6 and IL-8 concentrations rule out an EOS. In neonates with a low suspicion of EOS, low IL-6 and IL-8 concentrations postnatally can be used either not to start or to discontinue antibiotic treatment before results of a blood culture are definite.

Introduction

Early Onset Sepsis (EOS), defined as a sepsis starting within the first 2-3 days of life, is a severe and life-threatening disease. The mortality - when untreated - is around 100%. Specific perinatal risk factors, i.e., maternal fever or urinary tract infection, presence of chorioamnionitis and prolonged rupture of membranes, may increase the risk to develop an EOS [1].

EOS is difficult to diagnose because clinical symptoms are non-specific and may be subtle, and common laboratory parameters i.e., white blood cell (WBC) count, and C-reactive protein (CRP), are not sensitive enough for the diagnosis of EOS [2]. The gold standard to detect a neonatal sepsis is a blood culture. A positive blood culture is considered a definite proof of an infection, and a negative blood culture has a high negative predictive value [3]. Yet, the result of a blood culture is definite only after 48-72 hours. Given the difficulty to diagnose an EOS on the one hand, and the potential fatal consequences of delayed diagnosis and treatment on the other hand, it is recommended to start antibiotic therapy in all newborn infants with suspected EOS. This approach will cause, in retrospect, "unnecessary" antibiotic treatment in many infants. All these infants are treated with broad spectrum antibiotics for at least 2-3 days. These antibiotics are given intravenously and require that the infant is admitted at a pediatric ward. Inevitably, the wide-spread use of antibiotics has drawbacks. It will lead to the emergence of resistant bacteria [4], an abnormal colonisation of the gastro-intestinal tract, and carries the risk of yeast infections [5,6]. Although there is large variation in the incidence of multiresistent bacteria between several countries, the incidence has increased the previous decades and the use of antibiotics is considered a risk factor [7,8].

Cytokines, such as interleukin 6 and 8 are known to be involved in inflammatory processes and may be used to diagnose an EOS [9-11]. Cytokines are not yet used in clinical practice because sensitivity and specificity do not allow to differentiate between infants with and without a sepsis. Most studies evaluated levels of cytokines at the moment of suspected infection and 24 hrs later [12-14], whereas levels of IL-6 and IL-8 may decrease rapidly after the administration of antibiotics. Thereby studies might have missed the moment of the highest value of cytokines/chemokines. Secondly, previous studies combined infants with a

proven and suspected sepsis and compared these to infants without an infection, while it is also important to distinguish between infants with a suspected and proven sepsis. In this study we measured sequentially IL-6 and IL-8 during the first 48 hours of life in infants with a proven early onset sepsis, a suspected but not proven sepsis and controls.

Patients and Methods

During two and a half-years period (2007-2009) (pre)term infants born in participating hospitals and admitted to the neonatology ward because of suspected EOS were included after informed consent was obtained. Infants were not included when, at the time of admission, they were older than 6 hours or when multiple congenital abnormalities were seen. Infants were suspected of having an EOS when they showed at least one of following symptoms: requirement of any respiratory support (low flow, CPAP or mechanical ventilation) and/or supplemental oxygen, apnoea accompanied by either desaturation or persistent bradycardia (HR< 60 beats per minute); requirement of any circulatory support (vasopressors, crystalloids, hydrocortisone), tachycardia (HR>160 bpm), poor capillary perfusion; hyperirritability/lethargy, hypotonia, seizures; temperature instability (<36,6°C or > 38°C on two occasion within 24 hours); hyperglycemia (>10mmol/L) and metabolic acidosis (BE<-10 mmol/L). Laboratory investigation the moment of a suspected infection included complete white blood cell (WBC) count, CRP and blood culture. Other cultures were done only on indication. The blood culture was analyzed by the BACTEC method. Broad spectrum antibiotics were given to all suspected infants after septic screening and discontinued when the blood culture was negative at 72 hours in clinically stable infants. Guidelines for antibiotic treatment of EOS were not changed during the study period.

Cytokine measurements

At the moment of clinical suspicion of an infection and at 4-12, 12-36 and 36-60 hours thereafter 0,3 ml blood was taken for determination of levels of the cytokines IL-6 and IL-8 together with blood taking for clinical purposes. The blood was centrifuged, 50 µL of serum was taken and stored at minus

80°C within 15-30 min after blood collection. Frozen serum was shipped on dry ice to the laboratory of University Medical Center Groningen, The Netherlands where they were analyzed. Sera were thawed and analyzed using Invitrogen's Multiplex Bead Immunoassay. In a 96-well plate samples were prepared by adding beads of defined spectral properties which were conjugated to protein-specific capture antibodies, incubation buffer to bind cytokines to the protein-specific capture antibodies and biotinylated detector antibodies. Finally, streptavidin conjugated to the light-sensitive fluorescent protein R-Phycoerythrin was added and cytokine concentrations were analyzed with the Luminex detection system (Luminex Corp., Austin, Texas) using the programme StarStation 2.3. By monitoring the spectral properties of the beads and the amount of associated R-Phycoerythrin (RPE) fluorescence, the concentration of proteins was determined.

This prospective observational study was approved by the Medical Ethical Committees of all four participating hospitals. Two tertiary neonatal intensive care units, the University Medical Center Groningen, The Netherlands and the Harapan Kita Women and Children Hospital, Jakarta, Indonesia participated as well as two general hospitals, the Medical Center Leeuwarden and the Martini Hospital Groningen, The Netherlands.

Statistical analysis

Characteristics of the included infants were compared by the Fisher-exact test for categorical variables and by the Kruskal-Wallis test for continues variables, $p<0.05$ was considered significant and 95% confidence intervals were computed. We compared serum cytokine levels at all time points (0-4, 4-12, 12-36 and 36-60 hours) between all groups. Non-parametric test (Mann-Whitney U) from a computerised database (SPSS-11 for windows, SPSS Inc., 2001) was used to compare cytokines values between infants with proven versus suspected EOS and versus control infants.

Results

During the study period 510 newborn infants were admitted with the diagnosis suspected EOS. Six of these infants had a positive blood culture, yielding an incidence of 1.2 %. *Group B streptococci* (n=5) and *Escherichia*

coli (n=1) were cultured micro-organisms. Ten infants with a suspected sepsis but a negative culture were selected, matched for gestational age and birthweight, as well as 12 infants who showed maximally one sign of infection, had a negative culture and were included as controls. Clinical variables of all three groups are shown in Table 1.

Table 1. The Characteristics of study groups

	Control (n=12)	Clinical (n=9)	EOS (n=6)	Total (n=27)
Male	5	5	4	14
Vaginal delivery	6	3	5	14
Gestational age	36 (30-40)	32 (27 – 42)	36.5 (31-40)	35 (27-42)
Birth weight	2268 (960 – 4240)	1925 (1130 – 4500)	2935 (1895 – 3985)	2120 (960 – 4500)

GA and BW is median (minimum – maximum)

The pattern of IL-6 and IL-8 levels at the different time periods in the three groups is shown in fig. 1 and 2. IL-6 concentrations were significantly higher in the EOS group compared to control infants at all time periods ($p < 0.001$). IL-6 was higher in the EOS group compared infants with a clinical sepsis at all time points ($p< 0.05$), except at 4-12 hours, there was an overlap in levels however after the first 12 hours. Levels of IL-6 were only higher in the infants with clinical sepsis compared to controls during the first 12 hours. Levels of IL-8 were higher in the infants with EOS compared to controls up till 36 hours and in clinical sepsis up to 12 hours. Infants with EOS showed higher levels than clinical sepsis during the first 12 hours.

Figure 1. Serial measurements of IL-6 for infants suffering EOS and controls

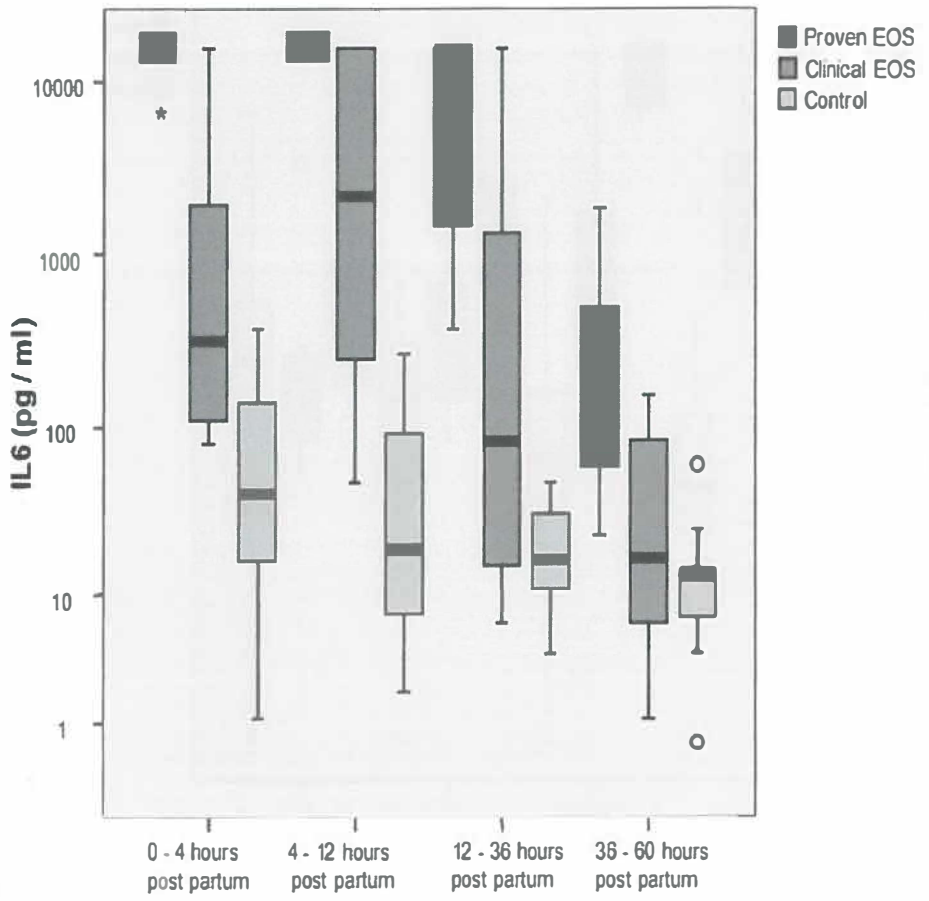
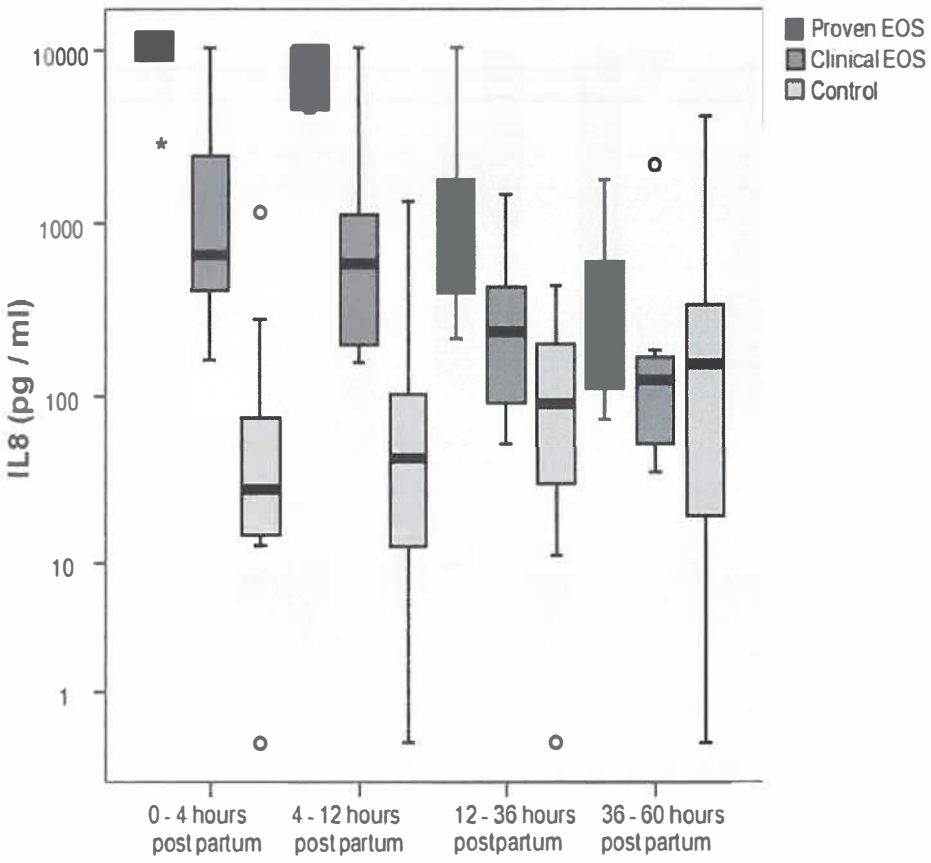


Figure 2. Serial measurements of IL-8 for infants suffering EOS and controls



Discussion

Our results show, for the first time in infants with a proven early onset sepsis that IL-6 is higher during a 48 hour period after the first symptoms of an infection compared to controls. Levels in infants with a suspected sepsis are also elevated, but decrease more rapidly compared to the group proven sepsis. Levels of IL-8 show the same pattern but decrease more rapidly after diagnosis. These results indicate that a high level of IL-6 indicates either a proven or suspected EOS, while a low level during the first 24 hours makes the presence of an infection and thereby the need to give antibiotics, very unlikely.

In this study, only in a minority of newborn infants who were screened for EOS postnatally an EOS was proven by a positive culture. These results are in line with previous data from Groningen and Jakarta [15,Chapter 5), Luck concluded that the majority of newborn infants screened and eventually treated for EOS do not show a positive culture [16]. The incidence of EOS is estimated to be 9/1000 neonatal admissions. Antibiotic are thus started unnecessary in the majority of newborn infants, which has several disadvantages. First, extensive use of antibiotics in newborn infants is related to the development of resistance to the antibiotics used [4], The antenatal administration of ampicillin to prevent Group B Streptococcus (GBS) infection in the mother and in the newborn infant shows a shift in bacteria causing an EOS, from GBS to ampicillin-resistent E.coli [17-19]. Waterer et al. showed that gram-negative bacteria, e.g., members of the Enterobacteriaceae family (E.coli, Enterobacter sp, Klebsiella, Citrobacter, Serratia sp.), are often resistant to at least one class of antibiotics used in newborn infants, including the β-lactams and aminoglycosides [20]. The development of resistance to antibiotics might even be a greater threat in developing countries [21-25]. In developing countries, gram-negative bacteria are the main cause for both early and late onset sepsis, and 45-85 % of bacteria are resistant to at least one or two antibiotics [26]. Other disadvantages of wide spread use of antibiotics include: i) an increased risk of opportunistic and fungal infections, especially in very low birth weight infants; ii) antibiotic-associated toxicity such as sensoneurinal hearing loss after gentamycine exposure, and iii) alteration of the natural microflora of the gastro intestinal tract, which may be related to immunological disorders.

Thus, the use of antibiotics should be restricted to newborn infants with a proven infection, and timely stopped in all other infants.

To date, the blood culture remains to be the gold standard to diagnose a neonatal sepsis. Studies in the past suggested that some infections might not have been detected because the blood culture was falsely negative [2]. The BACTEC system is the method presently used in most centers, and it gives reliable results after 24-48 hours [27]. The sensitivity and specificity of the blood culture is 100% when blood is taken using recommended procedures. Taking less than one ml of blood or not using aseptic methods can result in false negative or positive results [2]. Another problem, especially in developing countries is that the BACTEC system is not always available. ss

C-reactive protein (CRP), is world-wide used to detect or exclude a neonatal infection. Although an acute phase protein, peak CRP values are observed not as early as 24 hours after start of symptoms. A recent study found that the sensitivity and specificity of CRP to diagnose a sepsis is limited. In 93% of the infants with a proven sepsis was the CRP >10 mg/L [28]. Yet, CRP was also elevated in 73 % of the infants with a suspected sepsis. CRP was not elevated in 7 % of infants with a proven sepsis and in 27 % of the infants with a suspected infection [29]. The sensitivity of CRP as infection marker is increased when done serially within the first 24 hours after start of the symptoms [30]. Different cut-off values exist, but CRP levels above 10 mg/dL are considered compatible with an infection [28,30-32]. However, CRP is not a specific diagnostic parameter of an infection considering elevated CRP values in newborn infants with clinical sepsis, i.e. signs compatible with an infection but without a positive blood culture, in newborn infants with meconium aspiration, after perinatal asphyxia and post surgery. Procalcitonin (PCT) is another frequently used marker of infection. A recent meta analysis evaluating the use of PCT showed a sensitivity of 70-80 % and a specificity of 80-85 %, depending on the time after start of the symptoms [33].

Cytokines might be good markers to differentiate between newborn infants with a sepsis and newborn infants with the same clinical symptoms, not related to a bacterial infection. Cytokines studies in newborn infants with neonatal sepsis have been conducted since the late 1990s. Tumor necrosis

factor alpha, Interleukin-1, Interleukin-6 and Interleukin-8 were the first cytokines studied that showed higher levels in newborn infants with sepsis compared to controls [9,13,34,35]. These results indicated that newborn infants can produce cytokines. Further studies found that also GM-CSF, IL-6, IP-10 and IL-8 might be potential useful diagnostic infection markers in neonates, either alone or in combination with C-reactive protein or procalcitonin, either in cord blood or neonatal plasma [32,34-40]. Most studies included mainly or exclusively infants with a late onset sepsis. One study showed elevated levels of IL-8 and IP-10 in infants at the onset of an EOS, normal values were found at the second measurement at day four. In this study however infants with a proven and suspected or clinical infection were combined [10]. Another study showed elevated levels of IL-6 during the first 8 hours after start of clinical symptoms, between 16 and 24 hours only few of the infants showed elevated levels [11]. IL-8 was only elevated at the start of infection and 6 hours later while IL-10 was only elevated at the moment at the moment symptoms started. The difference with the results obtained by us can be explained that both studies mentioned included in the sepsis group both infants with a proven and suspected sepsis. We showed that IL-6 might decrease more rapidly in infants with a suspected or clinical sepsis compared to a proven sepsis. We found in our study in infants with a late onset sepsis that IL-6, IL-8 and IP-10 can not differentiate between infants with a proven vs a clinical sepsis [Chapter 7]. Taking these results together, we conclude that IL-6, IL-8 and IP-10 can differentiate between infants with a proven sepsis versus control infants, but not versus infants with a clinical infection. We showed in our study in infants with late onset sepsis that IL-15 and MIP1-a might be good markers to differentiate between proven and clinical sepsis [Chapter 7]. Fotopoulos however did not find increased levels of MIP1-a in infants with a sepsis on day one of life, but he included both infants with a proven and clinical or suspected sepsis [10]. More studies investigating these and other cytokines is needed to find the best cytokines that can help to differentiate between infants with a proven or clinical sepsis on day one.

Our results indicate that cytokines as IL-6 might be helpful to identify those patients where antibiotics can be stopped after 24 hours, so even before the result of the bloodculture is available. When IL-6 is not elevated at the

onset of symptoms and also not 24 hours later, it seems safe to stop antibiotics in these infants.

In conclusion, our data indicate that IL-6 is more elevated in infants with an EOS compared to a clinical sepsis, however there is overlap between groups, especially from 4 hours after the start of symptoms. Il-6 therefore cannot differentiate between infants with a proven and a clinical sepsis. At the same time, IL-6 can be used to identify infants with minor signs of infection who do not need antibiotics. Il-8 shows the same pattern as IL-6, with a more rapid decline after start of the symptoms.

References

1. Klinger G, Levy I, Sirota L et al. Epidemiology and risk factors for early onset sepsis among very-low-birthweight infants. Am J Obstet Gynecol. 2009 Jul;201(1):38.e1-6. Epub 2009 Apr 19.

2. Fowlie PW, Schmidt B. Diagnostic tests for bacterial infection from birth to 90 days:a systematic review. Arch Dis Child Fetal Neonatal Ed 1998;78:F92-8

3. Kaiser JR, Cassat JE, Lewno MJ. Should antibiotics be discontinued at 48 hours for negative late-onset sepsis evaluations in the neonatal intensive care unit? J Perinatol 2002;22(6):445-7.

4. de Man P, verhoeven BAN, Verbrugh HA et al. An antibiotic policy to prevent emergence of resistant bacilli. Lancet, 2000;355(18):973-8.

5. Saiman L, Ludington E, Pfaller M, Rangel-Frausto S, Wilbin RT, Dawson J, et al. Risk factors for candidemia in Neonatal Intensive Care Unit patients. The National Epidemiology of Mycosis Survey study group. Pediatr Infect Dis J 2000;19(4):319-24.

6. Fanaro S, Chierici R, Guerrini P, Vigi V. Intestinal microflora in early infancy: composition and development. Acta paediatr Suppl 2003;91(441):48-55.

7. Hyde TB, Hilger TM, Reingold A et al. Trends in incidence and antimicrobial resistance of early-onset sepsis: population-based surveillance in San Francisco and Atlanta. Pediatrics. 2002 Oct;110(4):690-5.

8. Lin CY, Hsu CH, Huang FY et al. The changing face of early-onset neonatal sepsis after the implementation of a maternal group B Streptococcus screening and intrapartum prophylaxis policy--a study in one medical center. Pediatr Neonatol. 2011 Apr;52(2):78-84. Epub 2011 Mar 16

9. De Bont, Martens A, van Raan S et al. TNFa,IL-1b and IL-6 Plasma Levels in Neonatal Sepsis.Pediatric Res,1992;380-3

10. Fotopoulos S, Mouchtouri A, Xanthou G et al. Inflammatory chemokine expression in the peripheral blood of neonates with perinatal asphyxia and perinatal or nosocomial infection. Acta Pediatr 2005;94(6):800-6.

11. Bender L, Thaarup J, Varming K et al. Early and Late markers for the detection of early onset neonatal sepsis. Danish Medical Bulletin, 2008; 55: 219-23

12. Groll AH,Meiser A,Weise M etal.Interleukin-6 as early mediator in neonatal sepsis.Pediatr Infect Dis J,1992;11(6): 496-8

13. Ng PC,Chui KM,Fok TF etal. Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecules, and C-reactive protein in preterm very low birthweight infant.Archives of Dis Childhood,1997;77:221-7

14. Damman O,Leviton A.Matenal Intrauterine Infection, Cytokine and Brain damage in the preterm newborn.Pediatr Res,1997;42(1):1-8.

15. Lusyati S, van den Broek P, Sauer PJ. Neonatal sepsis in a neonatal intensive care unit in Indonesia. J Hosp Infect 2009;71(4):383-5

16. Luck S, Torny M, d'Agapeyeff K et al. Estimated early-onset group B streptococcal neonatal disease. Lancet, 2003; Jun 7;361(9373):1953-4

17. Schraag S, Stoll B. Early onset neonatal sepsis in the era of widespread intrapartum chemopriphylaxis. Pediatr Infect Dis J 2006;25(10):939-40.

18. Stoll B, Hansen N, Fanaroff A et al. Changes in pathogen causing early onset sepsis in very low birth weight infants. N Eng J Med 2002;347(4):240-7.

19. Glasgow T, Young P, Wallin J et al. Association of intrapartum antibiotic exposure and late-onset serious bacterial infections in infants. Pediatrics 2005;116:696-702.

20. Waterer GW, Wunderink RG: Increasing threat of Gram-negative bacteria. Crit Care Med 2001;29:N75-N81(suppl).

21. Karthikeyan G, Premkumar K. Neonatal sepsis: Staphylococcus aureus as the predominant pathogen. Indian J Pediatr 2001;68:715–17.

22. Bhutta ZA, Yusuf K. Early-onset neonatal sepsis in Pakistan: a case control study of risk factors in a birth cohort. Am J Perinatol 1997; 14: 577–81.

23. Aurangzeb B, Hameed A: Neonatal sepsis in hospital-born babies: bacterial isolates and antibiotic susceptibility patterns. J Coll Physicians Surg Pak 2003;13:629-632.

24. Bell Y, Barton M, Thame M, et al. Neonatal sepsis in Jamaican neonates. Ann Trop Paediatr 2005;25:293-296.

25. Tiskumara R, Fakharee SH, Liu CQ et al. Neonatal infections in Asia. Arch Dis Child Fetal Neonatal Ed 2009 ;94(2):F144-8.

26. Kaistha N, Mehta M, Singla N et al. Neonatal septicemia isolates and resistance patterns in a tertiary care hospital of North India. J Infect Dev Ctries 2010;4(1):55-57.

27. Luke J, Mark WD, Joan F. Incubation time required for neonatal blood cultur become positive. J Paediatr Child Health 2006;42:797–802

28. Benitz W, Han M, Madan A et al. Serial serum CRP levels in the diagnosisof neonatal infection. Pediatrics 1998;102(4):E41.

29. Ohlin A, Björkqvist M, Montgomery SM et al. Clinical signs and CRP values associated with blood culture results in neonates evaluated for suspected sepsis. Acta Pædiatrica 2010; 99: 1635–1640.

30. Russel G, Smyth A, Cooke R et al. Receiver operating characterictics curve for comparison of serial neutrophyl band form and CRP in neonates at risk for infection. Arch Dis Child 1993;67:808-12.

31. Berger C,Uehlinger J, Ghelfi D et al. Comparison of CRP and white blood cell countwith differential in neonates at risk for neonatal septicaemia. Eur J Pediatr 1995;154:138-44.

32. Franz A, Steinbach G, Kron M et al. Reduction of unnecesary of antibiotic therapy in newborn infants using interleukin-8 and CRP as markers of bacterial infection. Pediatrics 1999;104:447-53.

33. Yu Z, Liu J, Sun Q et al.The accuracy of the procalcitonin test for the diagnosis of neonatal sepsis: A meta-analysis. Scandinavian Journal of Infectious Diseases 2010; 42: 723–733.

34. Kuster H, Weiss M, Willeitner AE et al. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestations.Lancet,1998; 352(9136):1271-7.

35. Ng PC, Li K, Wong RP et al. Proinflammatory and anti inflammatory cytokine responses in preterm infants with systemic infections. Arch Dis Child Fetal Neonatal Ed 2003; 88: F209-13.

36. Panero A, Pacifico L, Rossi N et al. Interleukin-6 in neonates with early and late onset infection.Pediatr Infect Dis J,1997;16(4):370-5.

37. Maciolek VM, Thijsen SF, Hemels M et al. Inflammatory mediators for the Diagnosis and Treatment of Sepsis in Early Infancy. Pediatric Res 2006;59:457-61.

38. Franz AR, Bauer K, Schalk A et al. Measurement of interleukin 8 in combination with C-reactive protein reduced unnecessary antibiotic therapy in newborn infants: a multicenter, randomized, controlled trial. Pediatrics 2004;114(6):1746.

39. Resch B, Gusenleitner W, Muller WD. Procalcitonin and interleukin-6 in the diagnosis of early-onset sepsis of the neonate. Acta Paediatr 2003;92(2):243-5.

40. Berner R, Niemeyer CM, Leititis JU et al. Plasma levels and gene expression of granulocyte colony-stimulating factor, tumor necrosis factor-alpha, interleukin (IL)-1beta, IL-6, IL-8, and soluble intercellular adhesion molecule-1 in neonatal early onset sepsis. Pediatr Res. 1998 Oct;44(4):469-77

CHAPTER 9

General Discussion

The fetus grows and develops in utero in an – almost – sterile environment. The infants become colonized by bacteria present in the genital tract during delivery. Further colonization takes place after birth, both from the mother and the environment. The colonization will not result in an infection as long as bacteria do not cross natural barriers, or, in case this might happen, the innate immune system cannot inactivate the micro-organisms before an infection develops. Skin and mucous membranes are the first barriers. When bacteria cross these barriers an infection might occur. Newborn infants who are admitted to a Neonatal Intensive Care Unit (NICU) have a high chance to be colonized with resistant bacteria. Colonization with these bacteria can lead to an infection, which rapidly can develop into a sepsis in newborn infants [1]. When the symptoms indicating an infection are detected in the first 72 hours of life, and the infection progresses into a sepsis, it is called "Early Onset Sepsis" (EOS). These infections are mostly caused by bacteria originating from the mother during the birth process, but colonization shortly after birth can also play a role, when the sepsis starts after the 3rd day of life, it is called "late onset sepsis" (LOS). These infections are also called acquired or nosocomial infections, as the origin of bacteria causing these infections is the environment in which the infant is cared for [1,2,3].

Two main risk factors to develop a sepsis are low gestational age and multiple–invasive procedures. In developed countries, where care is given according to high standards including aseptic measures, sterile products and careful hand washing, the rate of infections for children admitted in a NICU is relatively low with the exception of the very immature infants. Infants with long i.v. lines have a higher risk for a coagulase-negative staphylococcal infections compared to infants without these lines [4,5]. Recently, an increase in early onset sepsis due to Gram-negative bacteria was found in developed countries together with a decrease of sepsis caused by group B streptococci. This might be related to the administration of ampicillin to pregnant mothers who carry group B streptococci [2,6]. In developing countries the rate of infections is high at all gestational ages. Klebsiella sp., E. aerogenes, Pseudomonas, E.coli and Serratia sp. are the most common pathogens [7,8,9,10,11,12]. Secondly, these bacteria are mostly multi-drug-resistant. This alarming trend might be caused by high use of antibiotics in these countries. The potential sources of bacteria

between developed and developing countries are different. The long intravenous line is the potential source in developed countries, while in developing countries are contaminated infusions or devices, high use of reusable devices and lacking of hand hygiene [3,13].

Neonatal sepsis can be caused by viruses as well as bacteria. It is impossible to differentiate on clinical symptoms between viral and bacterial sepsis. A blood culture is the gold standard to detect a bacterial sepsis. The results of a blood culture are available only after 24-48 hours. The blood culture can be false-negative, for instance when not enough blood is taken [14]. In case of clinical signs compatible with infection clinicians will start antibiotics immediately, as a sepsis in a newborn infant can develop very rapidly with potentially a fatal outcome in a few hours. This rapid progression is most likely related to the not well-developed immune system [15,16]. Diagnostic markers that can differentiate at onset of symptoms between a bacterial infection and other factors causing the illness therefore are needed. Recently, a number of studies have evaluated cytokines, IL-6, IL-8, IL-10, IL-1, IP-10 and TNFα as markers of a bacterial infection in newborn infants (17-25]. All these cytokines were elevated in infants with a sepsis with or without a positive culture, compared to controls. An infection marker, however, should be able to differentiate between infants with a bacterial sepsis and infants with the same clinical symptoms due to another cause. Due to new technology, up to 25 cytokines now can be measured in only 25 µl plasma or serum. This makes it possible to study if any of those cytokines can differentiate between sick infants with bacterial sepsis vs. equally sick infants without a positive blood culture.

The neonatal intensive care unit of the Harapan Kita Woman and Children Hospital, Jakarta, Indonesia, is a third level intensive care unit. This unit is one of the referral hospitals in Indonesia. The research questions in this thesis are:

In this unit:

1. What is the incidence of neonatal sepsis, both early and late onset?
2. What are the types of bacteria causing both EOS and LOS?
3. Might solutions used to provide parenteral nutrition (TPN) and maintenance solutions be one of the causes of these infections and

can we prevent this by adapting the methods to prepare the TPN solutions?
4. What is the incidence of neonatal infections in a four-year period after the introduction of measures in the NICU to prevent infections?
5. How frequent are antibiotics prescribed in an infant admitted to the NICU?

In the NICU of the Beatrix Children's Hospital in Groningen, The Netherlands:

6. How frequent does an infection occur in an infant admitted to the NICU?
7. How frequent are antibiotics prescribed for a suspected sepsis?

Infection markers:

8. What are the levels of 25 cytokines during the first week of life in non-infected infants, and are the levels related to the gestational age?
9. Can we differentiate between infants with a culture proven LOS and those with clinical symptoms of an infection but a negative culture by measuring cytokines?
10. Can we differentiate between infants with a culture proven EOS and control infants by measuring cytokines ?

In Chapter 2 we describe that the incidence of early onset sepsis in the NICU of Harapan Kita Hosptal in 2003-2005 is relatively low and comparable to units in developed countries. Signs compatible with an infection were found in 133 out of 6600 life-born infants. Of these in only 9 infants a sepsis was proven (1,3 infants per 1000 live births or 4,1 infants per 100 admitted infants). All 133 suspected infants received antibiotics. These data are very comparable to the incidence of early-onset sepsis in the Beatrix Children's Hospital, UMCG, as shown in Chapter 5. We also found that the incidence of late-onset sepsis in Harapan Kita Hospital is very high. On day 3-5 a positive culture was observed in 63 of the 216

(29,2%) admitted infants, despite receiving antibiotics from birth. The incidence of infections was not related to gestational age or birth weight. EOS was most frequently caused by Serratia sp. and S. aureus, while Serratia sp., Klebsiella sp. and Enterobacteriaea were the main organisms causing LOS. Serratia sp. were the most prevalent bacteria in both EOS and LOS. Most bacteria were resistant to ampicillin, but not to the combination of ampicillin/sulbactam.

Serratia sp. as well as S. aureus, Klebsiella sp. and Enterobacter can be found in the gastro-intestinal tract. Serratia sp also can grow in solutions given intravenously to newborn infants. Studies have shown that Serratia sp. infections in human infants are almost always nosocomial infections, caused by colonization within the NICU and not from transmission from the mother. Arguments for nosocomial infections in our unit were the high incidence of infections on day 3-5 of life, and the finding that the incidence of infections was not related to the gestational age of the infants. We assumed therefore that our unit was highy contaminated with Serratia sp.. How long this situation already existed is unclear, as no data from before 2003 are available. One reason for the high incidence of Serratia sepsis on day 3-5 of life might be i.v. solutions contaminated with these bacteria, as shown in a study in a NICU in Mexico [26,27].

In Chapter 3 we show that the incidence of sepsis due to Serratia sp. was very high in infants who received an i.v. solution using a semi-open system after admission to our NICU. Infections with Serratia sp. were also observed in infants receiving only an i.v. solution and no other interventions. Based on these observations we decided to change the way i.v. solution were prepared and administered. First, the way the solutions were prepared was changed. Instead of preparing the solutions within the NICU, they were prepared under aseptic conditions in a separate room by two nurses who were dedicated to prepare these solutions. Secondly, only closed systems using a syringe were used. Solutions were prepared for maximally 24 hours, infusion lines were used for maximally 3 days. New solutions were prepared when changes in the i.v. fluids were necessary, It was not allowed anymore to inject solutions into the burette. The same mode of preparation was also used for total parenteral nutrition and medications. After changing the procedure to prepare and administer the

i.v. solutions we observed a sharp decrease in the incidence of sepsis. This was 3-5 times lower in the group receiving the new preparations and the closed system to deliver the solutions, compared to the previous situation. Especially the incidence of sepsis by Serratia sp. decreased significantly, from 31 of 37 positive cultures in a two-months period using the old system, to 4 of 5 positive cultures using the new set-up in the same time period. These results are compatible with our hypothesis phrased in Chapter 2, that the high rate of Serratia sp. sepsis in our unit was due to contamination of the environment, i.e., contaminated infusion was the main cause of sepsis. To prepare the i.v. solution in a separate room by dedicated personnel as well as the use of clinically closed systems to deliver the i.v. solutions is simple and not expensive. Therefore this practice can be followed in other NICUs in developing countries with a high rate of nosocomial infections. As interventions aimed to reduce the incidence of infections might have an effect, but the effect might not sustained, we followed prospectively the incidence of sepsis in the years thereafter.

Interventions aimed to reduce the incidence of nosocomial infections in a NICU are usually effective. The effect however might be temporary. Therefore, we investigated in Chapter 4 if the interventions as described in Chapter 3 reduced the incidence of nosocomial infections in a four-year period after making these interventions. We compared the incidence of late onset sepsis, bacteria causing these infections and the use of antibiotics between a two-year period before making these changes and a four-year period thereafter. All infants born in our hospital and admitted to our NICU were included. The incidence of late onset sepsis in admitted newborn infants decreased from 29,2% (63 of 216) in the first period to 9,1% (63 of 694) in the second period ($p<0.001$). The reduction was most pronounced in infants with a higher birth weight. In the first period, Serratia sp. were the main cause of infections, this almost disappeared in the second period. In 2010, the end of the second period, we observed an increase in infections due to S. epiderdimis.

The incidence of early onset sepsis showed no significant difference between both periods, although in the first period infections due to Serratia sp. were seen, and this was not observed anymore in the second period. Almost 80% of infants admitted to our NICU received antibiotics in both

time periods, and this was not related to gestational age. The duration of antibiotic therapy remained rather long, especially in infants with a suspected infection.

Our results show that the interventions as described in Chapter 3 did result in a sustained reduction in the incidence of nosocomial infections in our unit. Especially, infections due to Serratia sp. virtually disappeared. Still, the incidence of late onset sepsis in our unit is still higher compared to data published from developed countries. This might be due to a lack of awareness of the risk of infections by personnel entering the NICU. Hand hygiene might not always be optimal. The incidence of early onset sepsis in our unit is comparable to data from developed countries. The measures taken in our unit are simple and cost effective and can therefore be recommended to other units in developing countries with a similar high incidence of nosocomial infections as observed by us in 2003-2005.

In Chapter 5 we described the incidence of EOS in the NICU of the Beatrix Children's Hospital, UMCG, The Netherlands, in the period between January 2003 to December 2004 EOS was often suspected: 423 of 662 admitted infants were suspected suffering from an infection, and 423 of the infants received antibiotics. In only 10 infants the blood culture was positive: an incidence of 1,5/100 admitted infants. For one case of proven EOS 42 infants were treated with antibiotics. Antibiotic prescription was dependent on the gestational age. In the group of infants born after 24-27 weeks 68 infants received antibiotics for one case of proven sepsis compared to 21 in a group infants with a gestational age between 37-42 weeks. The high use of antibiotics for the suspicion of sepsis does not only happen in developed countries, and might even be more explicit in developing countries, as is shown in Chapter 4. Our results indicate that there is an important "overuse" of antibiotics in newborn infants. This is related to the fact that there is at present no good marker that can differentiate between proven and suspected sepsis. Further studies to find such markers are much needed.

Studies conducted in the past twenty years have indicated that cytokines might be good markers to detect a bacterial sepsis. IL-6, IL-8, IL-1, IL-10, IP-10 and TNFα have been studied in newborn infants. Levels of IL-6 and IL-1Ra already are increased before signs of an infection become apparent.

Still, the cytokines are not used in clinical practice. The cytokines were found to be different between infants with a proven sepsis and controls, but not to be able to differentiate with high sensitivity and specificity between sick infants with and without a proven bacterial sepsis. Recently, it became possible to measure 25 cytokines in 25 µl plasma or serum. Before testing if any of the above-mentioned cytokines can help to differentiate between proven and suspected sepsis we measured these cytokines in "healthy" newborn infants in the first week of life.

In Chapter 6 we show that almost all of the 25 tested cytokines were above the detection limit, in preterm as well as in term infants. A number of cytokines were more variable during the first four hours after birth compared to the period thereafter. IL-6 and IL-1-Ra were higher during the first four hours of life compared to the period thereafter. No trend over time was seen for almost all cytokines during the first week of life. IL-6 showed a negative trend during the first week of life in infants born after a gestation of more than 35 wks. We do not know if the higher levels of IL-6 and IL-1Ra immediately after birth are due to either physiological response or resuscitation with 100% oxygen (blended oxygen is not always available in every delivery room of our hospital) or other interventions just after birth. These possibilities were not evaluated in our study, but have been addressed previously. IL-6 and IL-1Ra can be increased due to other factors beyond an infection. This therefore indicates that IL-6 or IL-1Ra can not be used as a single marker for infection in neonates in the first days of life. A number of cytokines, especially those produced by T-cells such as IL-1Ra, IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IP-10, IFNγ, MIP-1a, MCP-1, and TNFα showed lower levels in preterm infants of 30-32 weeks compared to term infants in the first 72 hours of life.

In Chapter 7 we found that levels of 19 of 25 interleukins were higher in infants with proven late onset sepsis compared to non-infected patients. In infants with clinical symptoms of an infection, and a negative culture, the increase in cytokine levels was related to illness severity. In comparison with healthy infants, higher levels of 15 cytokines were found in infants with 3 or more clinical symptoms (severely ill infants) and higher levels of 8 cytokines were found in infants with "only" 2 symptoms (mildly ill infants). We found that IL-4, IL-5, IL-15, MIP-1a and MIP-1b could differentiate

between infants with a proven sepsis and infants with similar symptoms of illness, but a negative culture. Out of these five cytokines, IL-15 and MIP-1a showed a good diagnostic performance at 0 and 24 hours. When IL-15 and MIP-1a at 0 and 24 hours are not increased in severely ill infants above levels found in control infants, other causes than an infection should be looked for. In mildly ill infants with a negative culture, we did not find an increase in IL-6 levels. In contrast, Il-6 levels were increased in all infants with severe signs of illness, irrespective of the presence or absence of a bacterial infection. Thus, in mildly sick infants without an increase in IL-6 we believe that antibiotics can be stopped at 24 hours, without the need to wait for the results of the blood culture. Our results from Chapter 7 can help to reduce the frequent use of antibiotics in mildly ill infants in both developed and developing countries. In developing countries, especially in hospitals in remote areas in Indonesia, the results of a blood culture might not always be available within one week, due to the use of conventional methods. Measuring these cytokines might help to stop antibiotics earlier in these infants.

In chapter 8 we describe the first results of sequential measurement of the cytokines IL-6 and IL-8 in infants on the first two days of life. Three groups of infants were included, one group with a proven bacterial sepsis, one with comparable symptoms of disease but no positive blood culture, a group with only mild clinical symptoms and no positive blood culture served as control. We found that IL-6 is elevated in both groups with severe clinical signs of infection compared to the controls. Il-6 was elevated during the whole period in infants with a proven sepsis, and tended to decline faster in the group clinical sepsis. Il-8 also was elevated in both groups of sick infants but returned to control levels in approximately 24 hours. We conclude that the moment IL-6 is measured after the start of infection is not critical, and levels are elevated in both the proven and clinical septic infants. When IL-6 does not increase during the first 24 hours after start of clinical signs of an infection, this indicates the absence of a bacterial infection and antibiotics can be stopped.

In conclusion, in the NICU of Harapan Kita Hospital, Jakarta-Indonesia, the incidence of early onset sepsis is comparable to the incidence found in developed countries, but the rate of late onset sepsis is high. These

infections are mainly due to nosocomial acquired bacteria. Serratia sp. were the most predominant pathogen. After changing the system to prepare and administer i.v. solutions together with actively supporting alcohol based handrubs before and after touching an infant, the rate of infections significantly decreased. During the four-year period thereafter both the incidence and Serratia sp. remained at the same lower level. Bacteria causing a late onset sepsis changed, from Serratia sp. to multi-resistant S. epidermidis. S. epidermidis is also frequently cultured in infants with late onset sepsis in the NICU of the Beatrix Children's Hospital, UMC Groningen, The Netherlands.

At present no reliable markers to detect a sepsis in newborn infants are available; there is a clear overuse of antibiotics in these patients. There is a need therefore for new markers to differentiate between sick infants with and without a bacterial sepsis. Cytokines might be good markers to differentiate between these groups of infants, also in preterm infants. Preterm infants are also able to produce most cytokines, although at a slightly lower level. We found that IL-6 and IL-1Ra were higher at 4 hours after birth compared to the period thereafter. 14 Out of 25 tested cytokines were lower in "healthy" preterm infants of 30-32 weeks compared to infants of more than 36 weeks after 72 hours. We suggest that in future studies on cytokine levels in newborn infants gestational age should be considered as a confounding factor.

We showed that cytokines are able to differentiate between sick infants with a bacterial sepsis and infants with the same symptoms due to other causes. Especially MIP-1a and IL-15 are very promising markers. In our study these cytokines were elevated at the moment the infection was suspected and 24 hours later, in all newborn infants that had a bacterial infection. If MIP-1a and IL-15 are not increased in severely ill infants, other causes than a bacterial infection should be looked for. In mildly ill infants, when there is no increase in IL-6 at the first 24 hours after start of symptoms, the likelihood of an infection is very low, so antibiotics can be stopped at 24 hours.

References

1. Klein J and Remington JS. Current concept of infections of the fetus and newborn infant. In: Infectious diseases of the fetus and newborn infants. 1995, 4th ed.:1-19.

2. Stoll BJ, Hansen N, Fanaroff A et al. Late onset sepsis in very low birth weight neonates:the experience of the NICHD neonatal Research Network. Pediatrics 2002;110:285-91.

3. Zaidi A, Huskin C, Thaver D et al. Hospital-acquired neonatal infections in developing countries. Lancet 2005;365:1175-88.

4. Chien LY, Macnab Y, Aziz K et al. Variations in central venous catheter related infection risks among Canadian neonatal intensive care units. Pediatr Infect Dis J 2002;21(6):505-11.

5. Perlman S, Saiman L, Larson E. Risk factors for late onset health care associated bloodstream infections in patieents in neonatal intensive care unit. AJIC 2007; 35 (7): 177-82.

6. Azis K, McMilan DD, Andrews W, et al. Variations in rates of nosocomial infection among Canadian neonatal intensive care units may be practice-related. BMC 2005 (8); 5: 22-9.

7. Couto R, Carvallho E, Pedrosa T, et al. A 10-year prospective surveillance of nosocomial infections in neonatal intensive care units. Am J Inf Control 2007;35(3):183-89.

8. Jiang JH, Chiu NJ, Huang FY, et al. Neonatal sepsis in the neonatal intensive care unit:characteristics of early vs late onset sepsis. J Microbio Imunol Infect 2004;37:301-06.

9. Karthikeyan G, Premkumar K. Neonatal sepsis: Staphylococcus aureus as the predominant pathogen. Indian J Pediatr 2001;68:715–17.

10. Daoud AS, Abuekteish F, Obeidat A, et al. The changing face of neonatal septicaemia. Ann Trop Paediatr 1995;15:93–6.

11. Lim NL, Wong YH, Boo NY, et al. Bacteraemic infections in a neonatal intensive care unit: a nine months survey. Med J Malaysia 1995;50:59–63.

12. Nathoo KJ, Mason P, Chimbira TH. Neonatal septicaemia in Harare Hospital: aetiology and risk factors. Cent Afr J Med 1990;**36**:150–156.

13. Fanaroff A, Korones S, Wright L, et al. Incidence, presenting features, risk factor and significanceof late onset sepsis in very low birth weight infants. Ped Inf Dis J 1998; 17 (7): 1-10.

14. Luke J, Mark WD, Joan F. Incubation time required for neonatal blood cultur become positive. J Paediatri Child Health 2006; 42**:** 797–802.

15. Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev 1996;9:532–562.

16. Watkins LR, Maier SF, Goehler LE. Immune activation: the role of pro-inflammatory cytokines in inflammation, illness responses and pathological pain states. Pain 1995; 63:289–302.

17. Girardin EP, Berner ME, Grau GE, et al. Serum tumour necrosis factor in newborns at risk for infections. Eur J Pediatr 1990;149(9):645-7.

18. De Bont, Martens A, van Raan S, et al. TNFa,IL-1b and IL-6 Plasma Levels in Neonatal Sepsis. Pediatric Res 1992;380-3.

19. Buck C,Bundschu J,Bartmann P, et al. Interleukin 6:a Sensitive Parameter for the Early Diagnosis of Neonatal Bacteria Infection. Pediatrics,1994;93:54-8.

20. Ng PC,Chui KM,Fok TF, et al. Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecules, and C-reactive protein in preterm very low birthweight infant. Arch Dis Child 1997;77:221-7.

21. Ng PC, Li K, Wong RP, et al. Proinflammatory and anti inflammatory cytokine responses in preterm infants with systemic infections. Arch Dis Child Fetal Neonatal Ed 2003; 88: F209-13.

22. Panero A, Pacifico L, Rossi N, et al. Interleukin-6 in neonates with early and late onset infection. Pediatr Infect Dis J 1997;16(4):370-5.

23. Maciolek VM, Thijsen SF, Hemels M, et al. Inflammatory mediators for the Diagnosis and Treatment of Sepsis in Early Infancy. Pediatric Res 2006;59:457-61.

24. Ng PC, Li K, Chui KM et al. IP-10 is an Early Diagnostic Marker for Identification of Late Onset Bacterial Infection in Preterm Infants. Pediatric Res 2007;61:93-8.

25. Kuster H, Weiss M, Willeitner AE, et al. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestations. Lancet 1998; 352(9136):1271-7.

26. Macias A, Munos J, Herera L, et al. Nosocomial pediatric bacteriemia:the role of intravenous sset contaminaation in developing countries. Inf Control and Hosp Epidemiol 2004;25(3):226-30.

27. Macias A, Munos J, Galvan A, et al. Nosocomial bacteriemia in neonates related to poor standard of care. Pediatr Infect Dis J 2005;24(8):713-6.

Summary

Infections are an important cause of morbidity and mortality in newborn infants in developed, but even more so in developing countries. Symptoms seen in case of a suspected infection are not specific and also can be caused by other diseases. Infections in newborn infants rapidly can develop into a blood stream infection (sepsis) and cause death in a few hours. It is therefore needed to administer antibiotics immediately to all newborn infants who show signs that are compatible with an infection. This results in a frequent use of antibiotics in units for sick newborn infants (NICU) with as result the development of bacteria resistant against the most frequent used antibiotics.

In the **second chapter** of this thesis the incidence of infections within the NICU of the Harapan Kita Hospital, Jakarta, Indonesia is described. The incidence of infections in infants in the first 2-3 days of life was slightly higher compared to units in western countries, the incidence in later days was markedly higher. Infections occurring after the first days are almost all caused by bacteria acquired within the unit. In **chapter three** we show that the way solutions for intravenous use are prepared and administered is most likely an important cause for the high incidence of infections. With relative simple adaptations it was possible to achieve a marked reduction in the incidence of infections. As shown in **chapter four**, the lower incidence of infections was sustained, in the four years after making the changes did the incidence of infections stay at the lower level. The incidence however is still elevated compared with units in the western world. In **chapter five** we show that the incidence of infections in the neonatal intensive care unit of the Beatrix Children Hospital Groningen, The Netherlands, is comparable with units in other western countries. The use of antibiotics however is rather high, for one infant with a proven sepsis, 42 infants received antibiotics unnecessary. Better methods to differentiate between infants with and without a bacterial infection therefore is badly needed. Cytokines, small proteins that play an important role in the regulation of an infection, might be candidates. Cytokines however, might also be elevated by other causes of disease than an infection. In **chapter six** we show levels of 25 cytokines in newborn infants in the first week of life. A number of the cytokines were lower in infants born preterm compared to infants born after a normal pregnancy. These data can be used as reference data for studies in sick newborn infants. In **chapter seven** the same 25 cytokines are

measured in infants admitted to the NICU of the Harapan Kita Hospital, Jakarta, Indonesia. Three groups of infants were included, one group with a proven infection, one group with comparable severe clinical symptoms of disease but without a bacterial infection and one group with mild signs of disease and no infection. Eighteen of the 25 tested cytokines were elevated in the infants with clinical symptoms of disease compared to normal controls. The cytokines IL-6. IL-15 and MIP1-a are potentially good markers to confirm or disprove a bacterial infection in newborn infants. In **chapter eight** we show that IL-6 might be valuable to detect or disprove an infection in newborn infants in the first 2-3 days of life. IL-6 however was also elevated in infants with clinical signs of disease, not caused by a bacterial infection.

Conclusion. 1. Infections in newborn infants are an important problem, especially in less developed countries. 2. It is possible, with relative simple methods, to reduce the incidence of these infections. 3. Forty two newborn infants receive unnecessary antibiotics for one infant with a proven bacterial infection. 4. Cytokines, especially IL-6. IL-15 and MIP1-a, are potentially good markers to detect or disprove the presence of a bacterial infection.

Samenvatting

Infecties vormen een belangrijke oorzaak van ziekte en overlijden bij pasgeborenen in ontwikkelde, maar vooral in zich ontwikkelende landen. De symptomen die wijzen op een mogelijke infectie zijn niet specifiek en kunnen ook veroorzaakt worden door andere ziektes. Infecties bij pasgeborenen kunnen zeer snel leiden tot een bloedvergiftiging (sepsis) en binnen enkele uren tot de dood leiden. Daarom is het nodig bij alle pasgeborenen die symptomen passend bij een infectie vertonen direct antibiotica toe te dienen, wat leidt tot veelvuldig gebruik van antibiotica binnen afdelingen voor zieke pasgeborenen (NICU). Dit leidt vervolgens tot het opkomen van bacteriën die ongevoelig zijn voor veel gebruikte antibiotica.

In het **tweede hoofdstuk** van dit proefschrift is de incidentie van infecties binnen de NICU van het Harapan Kita Hospital. Jakarta, Indonesië beschreven. De incidentie van infecties gedurende de eerste drie levensdagen was iets hoger vergeleken met afdelingen in westerse landen, de incidentie in de periode daarna was sterk verhoogd. Infecties optredend na de eerste levensdagen zijn vrijwel altijd veroorzaakt door bacteriën aanwezig in de NICU. In **hoofdstuk drie** is aangetoond dat de wijze van prepareren en toedienen van infusie vloeistoffen waarschijnlijk een belangrijke oorzaak was voor de hoge incidentie van infecties. Met relatief eenvoudige maatregelen bleek het mogelijk het aantal infecties sterk terug te brengen. Zoals aangetoond in **hoofdstuk vier** was de daling in de incidentie van infecties blijvend, in de vier jaar na invoering van de maatregelen bleef de incidentie infecties op vergelijkbaar, veel lager nivo. Wel is de incidentie nog steeds iets hoger vergeleken met westerse landen. In **hoofdstuk vijf** is aangetoond dat de incidentie van infecties in de NICU van het Beatrix Kinderziekenhuis/UMCG vergelijkbaar is met die in andere westerse landen. Echter, het gebruik van antibiotica is zeer hoog, voor iedere pasgeborene met een aangetoonde infectie kregen 42 pasgeborenen onnodig antibiotica. Betere methoden om een onderscheid te maken tussen pasgeborenen met en zonder een bacteriële infectie is daarom noodzakelijk. Cytokines, kleine eiwitten die een belangrijke rol spelen in het reguleren van het ontstekingsproces, zouden dit onderscheid kunnen maken. Echter, cytokines kunnen ook verhoogd zijn door andere oorzaken van ziekte dan een infectie. In **hoofdstuk zes** is een overzicht gegeven van de gehaltes van 25 cytokines bij pasgeborenen zonder

infectie of ziekte in de eerste levensweek. Veel cytokines waren lager bij kinderen geboren na een korte zwangerschapsduur vergeleken met pasgeborenen geboren na een normale zwangerschap. Deze gegevens kunnen gebruikt worden als referentie waarden bij studies in zieke pasgeborenen. In **hoofdstuk zeven** zijn dezelfde cytokines gemeten bij pasgeborenen in de NICU van het Harapan Kita Ziekenhuis, Jakarta, Indonesia. Drie groepen kinderen werden geincludeerd, een groep mat een aangetoonde bacteriële infectie, een groep met vergelijkbaar ernstige verschijnselen maar zonder bacteriële infectie en een groep met lichte ziekte verschijnselen. Achttien van de 25 onderzochte cytokines waren verhoogd in de kinderen met ziekteverschijnselen vergeleken met kinderen zonder verschijnselen. De cytokines IL-6, IL-15 en MIP1-a zijn potentieel goede markers voor het aantonen of uitsluiten van een bacteriële infectie bij pasgeborenen. In **hoofdstuk acht** is aangetoond dat IL-6 van waarde kan zijn bij het aantonen of uitsluiten van een bacteriële infectie bij een pasgeborene in de eerste levensdagen. Echter, IL-6 was ook verhoogd bij pasgeborenen met ziekteverschijnselen niet veroorzaakt door een bacteriële infectie.

Conclusie. 1.Infecties bij pasgeboren vormen een groot probleem, zeker in minder ontwikkelde landen. 2. Met relatief eenvoudige maatregelen is het mogelijk de incidentie van infecties te verminderen. 3. Voor een pasgeborene met een aangetoonde bacteriële infectie krijgen 42 pasgeborenen onnodig antibiotica. 4. Cytokines, met name IL-6, IL-15 en MIP1-a zijn potentiiel goede markers om een bacteriële infectie aan te tonen of uit te sluiten.

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Now, after a period, I am here today with this thesis. Even though my performance in doing these studies might not yet be so well. But at least it started .....

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Setyadewi Lusyati, Jakarta, June 2011

Curriculum vitae

Setyadewi Lusyati was born at June 13th-1966, at the Lafayette Hospital-Malang. She grew up and lived in Lawang until her graduation from senior high school (1984). Then she started study medicine at the Medical School Airlangga University in Surabaya,East-Java (1984-1990). Just after her graduation, she went to East-Timor, to work at the Central Health District – Liquisa-East Timor (1991-1993). To work outside of Java is called Wajib Kerja Sarjana (WKS), part of the conditions to become an Indonesia goverment employee. In 1991 she became an Indonesia goverment employee (pegawai Negeri Sipil). Therafter she studied Pediatrics at The Pediatric Department Padjajaran University in Bandung (1994-1999). After became Pediatrician, again she worked at Ende Hospital-Flores-East Nusa Tenggara, the place which famous with Kelimutu Lake and Komodo Island. She set up a Neonatal Unit (High Care) there. In the fall of 2001 she and her family decided to return back to Jakarta, then she was accepted to join the Pediatric team at the Harapan Kita Women and Children Hospital,Jakarta (middle of 2002).

From September 2004 till July 2005 she received additional training in neonatology at the Beatrix Children's Hospital-University Medical Center Groningen,Groningen-The Netherlands under supervision of Prof. Dr. Arend F. Bos and Prof.Dr. Pieter J. Sauer. She started research on neonatal infection at the NICU of UMCG after receiving a grant from Nestle Nutrition Institute (May 2005). The first study was on the incidence of neonatal sepsis, bacteria causing these infections, and the use of antibiotics in the NICU of the Beatrix Children Hospital. Soon after-returning from Groningen to Jakarta she continued her studies with the same topic at the NICU of Harapan Kita Hospital. In July 2007 she was awarded a grant for doctoral research from the NUFFIC (Netherlands Organization for International Cooperation in Higher Education) for period of 2007-2012. With NUFFIC support she worked as a Doctoral Research Fellow, in a collaboration of the Beatrix Children Hospital/UMCG Groningen and Harapan Kita Hospital under supervision of Prof. Dr. Pieter J. Sauer. Since August 2005 she works as a Pediatrician-Neonatologist at Harapan Kita Women and Children Hospital Jakarta.

Setyadewi Lusyati is also a mother from two daughters, Aprilia Utami Setya Wijoyo (18 years) and Dhia Lintang Setya Wijoyo (12 years).